



WALLAGA UNIVERSITY
SCHOOL OF GRADUTE STUDIES

**GREEN SYNTHESIS OF Ag-Ni CO-DOPED ZnO
NANOCOMPOSITES USING *MANGIFERA INDICA* PLANT
LEAF EXTRACT FOR ENHANCED ANTIOXIDANT AND
ANTIBACTERIAL ACTIVITIES**

MSc. THESIS

By: DESSALEGN BABSA

MAY 2025

NEKEMTE, ETHIOPIA



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**A Thesis Submitted to the College of Natural and Computational Sciences in
Partial Fulfillment of the Requirements for the Degree of Masters of Science
in Inorganic Chemistry**

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As members of the Board of Examining of the Final MSc. thesis open defence, we certify that we have read and evaluated the thesis prepared by **Dessaegn Babsa Oljira** under the title **“Green synthesis of Ag-Ni co-doped ZnO nanocomposites using *Mangifera indica* plant leaf extract for enhanced Antioxidant and Antibacterial activities”** and recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Inorganic Chemistry.

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I hereby certify that all the corrections and recommendations suggested by the board of examiners are incorporated into the final thesis entitled **“Green synthesis of Ag-Ni co-doped ZnO nanocomposites using *Mangifera indica* plant leaf extract for enhanced Antioxidant and Antibacterial activities”** by Dessaegn Babsa Oljira.

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DECLARATION

I declare here by that this thesis entitled “**Green synthesis of Ag-Ni co-doped ZnO nanocomposites using *Mangifera indica* plant leaf extract for enhanced Antioxidant and Antibacterial activities**” is the true reflection of my student’s research and accepted in partial fulfilment of the requirement for the award of the degree of masters of science in inorganic chemistry by the school of graduate studies, Wallaga University through the college of natural and computational sciences. Dessalegn Babsa Oljira completed this thesis under my supervision and guidance. Any part of this thesis has not been submitted for a degree in any other institution of higher education.

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I, Mr. Dessalegn Babsa, hereby declare and affirm that the thesis entitled “**Green synthesis of Ag-Ni co-doped ZnO nanocomposites using *Mangifera indica* plant leaf extract for enhanced Antioxidant and Antibacterial activities**” is my own work conducted under the supervision of Raji Feyisa (PhD). I have followed all the ethical principles of scholarship in the preparation, data collection, data analysis, and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I have adequately cited and referenced all the original sources. I also declare that I have adhered to all principles of academic honesty and integrity, and I have not misrepresented, fabricated, or falsified any idea, data, fact, or source in my submission. This thesis is submitted in partial fulfilment of the requirement for a degree from the Postgraduate Studies program at Wallaga University. I further declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS AND ACRONYMS

DMSO	:	Dimethyl Sulfoxide
DPPH	:	2, 2-diphenyl-1-picrylhydrazyl
DPPH-H	:	2, 2-diphenyl-1-picrylhydrazine
FCC	:	Face-Centered Cubic
FT-IR	:	Fourier Transform Infrared
IC₅₀	:	Half Maximal Inhibitory Concentration
Mev	:	Milli Electron Volt
MHA	:	Mueller Hinton Agar
NCs	:	Nanocomposites
NPs	:	Nanoparticles
ROS	:	Reactive Oxygen Species
RSA	:	Radical Scavenging Activity
RTFM	:	Room Temperature Ferromagnetism
UV-Vis	:	Ultraviolet-Visible Spectroscopy
XRD	:	X-ray Diffraction

TABLE OF CONTENTS

ACKNOWLEDGEMENT.....	v
LIST OF ABBREVIATIONS AND ACRONYMS	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF SCHEMES	xiii
LIST OF APPENDICES	xiv
ABSTRACT.....	xv
1. INTRODUCTION.....	1
1.1. BACKGROUND OF THE STUDY.....	1
1.3. Objectives of the study	5
1.3.1 General objective.....	5
1.3.2. Specific objectives.....	5
1.4 The Significance of the study	5
2. LITERATURE REVIEW	7
2.1. Metal oxide Nanoparticles.....	7
2.2. Monodoped metal Oxide Nanocomposites.....	10
2.3. Codoped metal Oxide Nanocomposites	12
2.4. Strategies of Nanoparticles and nanocomposite Synthesis.....	13
2.5. Factor affecting green synthesis of nanomaterials	15
2.5.1. Effect of Concentration of Metal Precursors.....	16
2.5.2. Effect of volume of plant extracts	16
2.5.3. Effect of Reaction Temperature	16
2.5.4 Effect of pH.....	17
2.6. Plant-mediated synthesis of nanoparticles and nanocomposites	17
2.7. <i>Mangifera Indica</i> plant	18
2.7.1. <i>Mangifera indica</i> Leaf Constituents.....	20
2.7.2. Antioxidant and Antibacterial Benefits of <i>Mangifera Indica</i> Leaf Extract	21
2.8. Characterization technique for nanoparticles and nanocomposites.....	22
2.8.1. UV-Visible spectroscopy	22

2.8.2. Fourier Transformation Infrared (FT-IR) Spectroscopy	22
2.8.3. X-ray diffraction (XRD) Powder	23
2.9. Antioxidant and Antibacterial Activities of Nanoparticles and Nanocomposites	24
2.9.1. Antioxidant activity of nanoparticles and nanocomposites.....	24
2.9.2. Antibacterial activity of nanoparticles and nanocomposites.....	28
3. MATERIALS AND METHODS	30
3.1. Chemicals and Reagents	30
3.2. Instrument, Apparatus, and Equipment	30
3.3. Collection and Preparation of <i>Mangifera</i> Indica Leaf Extract	30
3.4. Phytochemical Analysis of <i>Mangifera indica</i> Leaf Extract	31
3.5. Some Optimization Parameters for monodoped and codoped nanocomposites using <i>Mangifera indica</i> Leaf Extract.	32
3.6. Synthesis of nanoparticles and nanocomposites using <i>Mangifera indica</i> leaves extract. ..	34
3.6.1. Green Synthesis of ZnO NPs	34
3.6.2. Green Synthesis of Ag-ZnO NCs.....	34
3.6.3. Green Synthesis of Ni-ZnO NCs.....	35
3.6.4. Green Synthesis of Ag-Ni/ZnO NCs.....	36
3.7. Characterizations of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs using <i>Mangifera indica</i> leaf extract.	37
3.7.1. UV-Vis Analysis	37
3.7.2. FT-IR Analysis	38
3.7.3. XRD Analysis	38
3.8. Antioxidant and Antibacterial test of nanoparticles and nanocomposites using <i>Mangifera indica</i> leaf extracts.....	39
3.8.1. Antioxidant Activity Test.....	39
3.8.2. Anti-bacterial Activity Test.....	40
4. RESULTS AND DISCUSSIONS.....	42
4.1. Phytochemical analysis of <i>Mangifera indica</i> leaves extract	42
4.2. Optimization of reaction conditions.	43
4.2.1. Concentration of precursor.....	43
4.2.2. Varying volumes of plant extract	45
4.2.3. Effect of Temperature.	47
4.2.4. Effect of pH.....	48
4.3. Synthesized NPs and NCs using <i>Mangifera indica</i> Leaf Extract.	50
4.4. Characterization of nanoparticle and nanocomposites.	52

4.4.1. Electronic spectra of the synthesized nanoparticles (UV-Vis spectroscopy).....	52
4.4.2. Fourier transforms infrared (FT-IR) spectroscopy.....	55
4.4.3. X-ray diffraction (XRD) analysis of nanoparticles and nanocomposites.	59
4.5. Antioxidant activity of nanoparticles and nanocomposites using <i>Mangifera indica</i> leaf extract.	63
4.6. Antibacterial Activity of nanoparticles and nanocomposites using <i>Mangifera indica</i> leaf extract.	71
4.7. Possible antibacterial mechanisms of nanoparticles and nanocomposites.	78
5. CONCLUSION AND RECOMMENDATION	80
5.1. Conclusion.....	80
5.2. Recommendation.....	82
6. REFERENCES	83
7. APPENDICES	97

LIST OF TABLES

Table 1. Summary of plant-mediated metal-metal codoped metal oxide nanocomposites with their applications.	13
Table 2. Taxonomical Classification of <i>Mangifera indica</i> plant	19
Table 3. Phytochemical screening of the plant extracts.....	42
Table 4. Fourier transforms infrared spectra for ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, 2% Ag-Ni/ZnO NCs, 4% Ag-Ni/ZnO NCs, 6% Ag-Ni/ZnO NCs and <i>Mangifera indica</i> leaf extract.	57
Table 5. FWHM, diffraction angle, and average crystalline size of <i>Mangifera indica</i> leaf extract-mediated ZnO NPs, Ni-ZnO NCs, and Ag-ZnO NCs.....	62
Table 6. FWHM, diffraction angle, and average crystalline size of Ag-Ni/ZnO NCs (2%, 4%, and 6%).	62
Table 7. The DPPH radical scavenging activity of the <i>Mangifera indica</i> leaf extract, ZnO NPs, Ni-ZnO NCs, Ag-ZnO NCs and ascorbic acid.	64
Table 8. The DPPH radical scavenging activity of Ag-Ni/ZnO NCs (2%, 4%, 6%) and ascorbic acid.....	65
Table 9. Comparing the effectiveness of the current study with some reported antioxidant activity of green synthesized NPs and NCs.	70
Table 10. Zone of inhibition of synthesized ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs (2%, 4%, and 6%) using <i>Mangifera indica</i> leaf extract against <i>S. aureus</i> and <i>E. coli</i>	72
Table 11. Comparing the current study with some reported antibacterial activity of green synthesized NPs and NCs.	77

LIST OF FIGURES

Figure 1. Various methods for the synthesis of NCs.	15
Figure 2. Image of <i>Mangifera indica</i> plant.	20
Figure 3. Structure of Phenolic compounds found in <i>Mangifera indica</i> plant extract.	20
Figure 4. Concentration optimization of NPs and NCs.	44
Figure 5. Optimization of volume <i>Mangifera indica</i> leaf extract for NPs and NCs.	46
Figure 6. Temperature optimization of NPs and NCs	47
Figure 7. pH optimization of NPs and NCs	49
Figure 8. UV-Vis spectra of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, 2% Ag-Ni/ZnO NCs, 4% Ag-Ni/ZnO NCs and 6% Ag-Ni/ZnO NCs.	54
Figure 9. UV-Vis spectrum of <i>Mangifera Indica</i> leaf extract.	54
Figure 10. Optical bandgap of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs (2%, 4%, and 6%).	55
Figure 11. FTIR spectral absorption band of NPs and NCs (a) and <i>Mangifera indica</i> leaf extract (b).	58
Figure 12. XRD pattern of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs (2%, 4% and 6%).	63
Figure 13. Bar graph representations of A) IC_{50} value and B) % RSA for <i>Mangifera indica</i> , ZnO NPs, Ni-ZnO NCs, Ag-ZnO NCs and Ag-Ni/ZnO NCs (2%, 4% and 6%) and ascorbic acid.	66
Figure 14. DPPH radical scavenging activity curve for <i>Mangifera indica</i> extract, ZnO NPs, Ni-ZnO NCs, Ag-ZnO NCs, Ag-Ni/ZnO NCs (2%, 4%, and 6%), and ascorbic acid.	68
Figure 15. Picture showing zone of inhibition for the antibacterial activities of <i>Mangifera indica</i> leaf extract-mediated synthesized ZnO NPs(a), Ag-ZnO NCs(b), Ni-ZnO NCs(c), and Ag-Ni/ZnO NCs (2%(d), 4%(e), and 6%(f)).	74
Figure 16. Bar graph representation of antibacterial activity for ZnO NPs, Ni-ZnO NCs, Ag-ZnO NCs, and Ag-Ni/ZnO NCs (2%, 4%, and 6%) of A) <i>S. aureus</i> and B) <i>E. coli</i>	76

Figure 17. Proposed Antibacterial inhibition mechanism of Ag-Ni/ZnO NCs.	78
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LIST OF SCHEMES

Scheme 1. Mechanisms of antioxidants activity.....	27
Scheme 2. Extraction of <i>Mangifera indica</i> leaves extract flow chart.....	31
Scheme 3. The synthesis method flow chart of ZnO NCs.....	34
Scheme 4. The synthesis method flow chart of Ag-ZnO NCs.....	35
Scheme 5. The synthesis method flow chart of Ni-ZnO NCs.....	36
Scheme 6. The synthesis method flow chart of Ag-Ni/ZnO NCs.....	37
Scheme 7. Schematic flow chart of antioxidant analysis.....	40
Scheme 8. Mechanism for synthesis and stabilization of Ag-Ni/ZnO NCs using <i>Mangifera indica</i> leaf extract.....	51

LIST OF APPENDICES

Appendix 1. Percent by mass and molarity of dopant (nickel nitrate hexahydrate and silver nitrate) with zinc sulfate heptahydrates by using calculated volume ratio method.	97
Appendix 2. Preparation process of <i>Mangifera indica</i> leaf extract.	97
Appendix 3. Phytochemical screening test results for flavonoid (a), alkaloid (b), tannin(c), saponin (d) and phenol (e) of <i>Mangifera indica</i> leaf extract.	98
Appendix 4. Figurative description of synthesized ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs and Ag-Ni/ZnO NCs (2%, 4% and 6%).	98
Appendix 5. FTIR Spectral absorption band of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, 2% Ag-Ni/ZnO NCs and <i>Mangifera indica</i> leaf extract.	99
Appendix 6. FTIR Spectral absorption band of 4% Ag-Ni/ZnO NCs, 6% Ag-Ni/ZnO NCs and <i>Mangifera indica</i> leaf extract.	100
Appendix 7. XRD graphs of synthesized Ag-ZnO NCs and 6% Ag-Ni/ZnO NCs	101

ABSTRACT

Doping, which involves introducing impurity atoms into bioactive metal oxide nanoparticle to modify their structural, optical, electrical, and biological properties, is a well-established method. Currently, the most pressing challenges are chronic diseases caused by free radicals and microbial caused diseases that are very resistant to medication. This study describes the biosynthesis of Ag-Ni/ZnO nanocomposites using *Mangifera indica* leaf extract for investigating their antioxidant and antibacterial activities. The synthesized nanoparticles were characterized using UV-Visible absorption (UV-Vis), powder X-ray diffraction (PXRD), and Fourier transform infrared (FTIR) spectroscopy. Ultraviolet-visible spectral data were 360, 362, and 366 nm, with corresponding band gap energy 3.02, 2.92, and 2.72 eV for 2% Ag-Ni/ZnO, 4% Ag-Ni/ZnO, and 6% Ag-Ni/ZnO NCs, respectively. As the dopant ratio increased, the absorption wavelength also increased, due to the incorporation of Ag and Ni ions into the lattice. FTIR spectra also revealed that the metal-oxygen bond (M-O) in NPs and NCs and phytochemicals in the plant extract act as capping and stabilizing agents for the biogenically Ag-Ni codoped ZnO NCs. From X-ray crystallography data, ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs and Ag-Ni/ZnO NCs show hexagonal wurtzite crystal structure and trend in size follows the order: ZnO NPs < Ni-ZnO NCs < Ag-ZnO NCs < 2 % Ag-Ni/ZnO NCs < 4 % Ag-Ni/ZnO NCs < 6 % Ag-Ni/ZnO NCs. The crystalline size was ranged between 18.51 - 25.13 nm. The three additional diffraction peaks (111), (200), and (220) belong to the face-centered cubic (fcc) structure of silver in Ag-ZnO NCs. The study investigated the radical-scavenging activity of a synthesized nanoparticles and nanocomposites at different concentrations. The synthesized ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs and Ag-Ni/ZnO NCs using *Mangifera indica* leaf extract demonstrated significant antioxidant activity, with %RSA values of 67.02 ± 0.00 , 81.75 ± 0.00 , 77.57 ± 0.00 and 92.36 ± 0.00 respectively. The antioxidant activity of Ag-Ni/ZnO NCs was significantly greater than that of pure ZnO NPs, attributed to synergistic effects, bioactive compound presence, altered electronic properties, improved stability, and dispersion. At higher concentrations, ZnO NPs inhibit 15.6 ± 0.00 mm *S. aureus*, Ag-ZnO NCs inhibit 21.00 ± 0.80 mm *S. aureus*, Ni-ZnO NCs inhibit 16.30 ± 0.41 mm *E. coli*, and 6 % Ag-Ni/ZnO NCs inhibit 23.42 ± 0.40 *S. aureus*. The highest inhibition zone (23.41 ± 0.40 mm) was found for 6% Ag-Ni/ZnO nanocomposites against *S. aureus*. ZnO NPs, Ag-ZnO NCs, and Ag-Ni/ZnO NCs were more effective against Gram-positive bacteria than gram-negative bacteria due to structural difference between them. The results showed that the green-synthesized monodoped and codoped NCs show superior antioxidant and antibacterial activities compared to pure ZnO NPs due to synergetic effect of codopant and the presence of bioactive compound in *Mangifera indica* leaf extract.

Keywords: Ag-Ni/ZnO nanocomposites; Green synthesis; *Mangifera indica*; antioxidant; antibacterial activity

1. INTRODUCTION

1.1. BACKGROUND OF THE STUDY

Nanotechnology is a field of science and engineering that focuses on the study and manipulation of nanomaterials with at least one dimension ranging from one to one hundred nanometers. Nanomaterials have attracted a lot of interest in recent decades because of their intriguing characteristics and prospective uses in a wide range of industries. Because these materials' properties change a lot depending on their size and shape, scientists are interested in making semiconductor nanoparticles with different shapes, such as nanoflowers, nanospheres, nanoplates, and nanobelts and nanowires (Iqbal *et al.*, 2019). A variety of nanomaterial classes are being extensively explored, one of which is transition metal oxides. However, the synthesis techniques used have a significant impact on how metal oxide nanocomposites are applied. The production of nanoparticles can be accomplished by three basic methods: chemical, biological, and physical. Both physical and chemical methods to reduce and cap nanoparticles need a lot of energy and use hazardous chemicals that stay on their surfaces even after several washings. Therefore, they cannot be employed in biomedicine due to the potential of toxicity (Haq *et al.*, 2020).

In order to overcome the hazardous effects and limitations of the chemical and physical approach, the biological synthesis of NPs and NCs has gained more importance recently. A plant-based approach, known as the "green synthesis method," is robust, biodegradable, biocompatible, environmentally friendly, and economically feasible, and it uses nontoxic, nonhazardous solvents (Gour and Jain, 2019). Because plants are easily obtainable, yield more, and contain a variety of phytochemicals that may quickly reduce a range of metal oxides and produce NCs, there has been a substantial amount of research into using different plant parts for NCs synthesis (Khan *et al.*, 2021). By absorbing on the surface of the nanoparticles during the particle formation processes, the secondary metabolites found in plant extracts, such as phenols, alkaloids, saponins, tannins, flavonoids, and terpenoids, act as both stabilizing and reducing agents. This ultimately results in the occurrence of subsequent surface effects during their application in the formation of NCs (Suwan *et al.*, 2019).

Nanoparticles and nanocomposites are made from a variety of plant extracts. For example, ZnO NPs with extract from *Mangifera indica* seeds have been used for medical applications and their photocatalysis (Rajeshkumar *et al.*, 2023); Se-ZnO NCs with extract from *Mangifera indica* leaves for photodegradation and antibacterial applications (Dhivya *et al.*, 2019); Ag-Au/ZnO NCs with extract from *Justicia adhatoda* leaves for antibacterial and anticancer activities (Pandiyar *et al.*, 2019); and Ag-Cu/ZnO NCs with extract from *Aloe vera* leaves with antibacterial activities (Rashid *et al.*, 2024).

These days, the distinctive properties of metal oxide nanoparticles and their composites, such as their homogeneous morphology and capacity for light absorption, make them extremely important. The domains of biomedicine, bioimaging, and sensors are seeing an increase in their daily use. Anti-inflammatory, antibacterial, anticancer, antioxidant, antimutagenic, and antidiabetic properties are possessed by metal oxide nanoparticles and nanoclusters (Iqbal *et al.*, 2019). Because of its exceptional qualities, which include low toxicity, low ecological effect, and biocompatibility, transition metal oxide nanomaterials (NCs) are gaining immense appeal (Tabrez *et al.*, 2022).

Zinc Oxide (ZnO) has garnered attention among other metal oxides because of its distinct chemical, physical, and biological characteristics (Mohammadi *et al.*, 2018). It is an n-type semiconductor that is similar to TiO₂ in terms of mode of action, band gap (3.37 eV), and high exciton binding energy (60 meV). However, the quick recombination of charge carriers, low quantum yields, poor photostability, poor adsorption ability, and restricted reusability are the main drawbacks of ZnO as a photocatalyst (Kumar *et al.*, 2017). Researchers have shown a great deal of interest in transition metal oxides acting as hosts, specifically zinc oxide (ZnO), copper oxide (CuO), iron oxide (Fe₂O₃), TiO₂, SnO₂, etc. (Karthikeyan *et al.*, 2023). Doping, which involves introducing impurity atoms into metal oxide semiconductors to alter their optical characteristics, is a well-established method (Mostafa *et al.*, 2020). Metal doping is a successful method that has attracted a lot of interest for adjusting the optoelectronic characteristics of the materials and doping of ZnO makes it more absorbent of light in the visible spectrum. To enhance the optical characteristics of ZnO, a range of transition and non-transition metals have been doped into it. Regarding monodoped ZnO NCs, various studies have been reported: Several scientists reported that Ag-ZnO significantly improves the photocatalytic, antibacterial, and anticancer activity of ZnO (Gudkov *et al.*, 2021).

Ag acts as an electron donor in this system, reducing the recombination of electrons in ZnO lattices, while Ni lowers charge carrier recombination by imparting a trap state (Vignesh *et al.*, 2018). By creating an impurity level between the valence band and conduction band, nickel allows for the fine-tuning of optical characteristics. Co-doped semiconductor materials have received more attention as a means of eliminating undesirable states and enhancing semiconductor efficiency. Furthermore, by creating trap states, codoping modifies the optical, electrical, and magnetic characteristics, improving charge separation and band gap tuning (Reddy *et al.*, 2020). This can increase visible light absorption and decrease charge carrier recombination, and since codoping provides superior optoelectronic performance than single metal doping, it has attracted a lot of interest. Recently, Mn-Cu/ZnO NCs using *Corn silk* for Antimicrobial and photocatalytic activities (Wali *et al.*, 2022), Ag-Au/ZnO NCs greenly by using *Justicia adhatoda* leaves for Antibacterial and Anticancer activities (Pandiyani *et al.*, 2019) and Ag-Cu/ZnO NCs using *Aloe vera* leaf for Antibacterial activities (Rashid *et al.*, 2024) have been reported. Because of their simple permeability, and rapid transport, scientists are creating nanoparticles as a viable substitute for traditional medications. Given that green approaches are the most effective in biomedical applications and that many nanoparticles are being manufactured and investigated for their antioxidant and antimicrobial qualities, it is imperative to comprehend the antimicrobial effects of produced NPs and NCs (Qamar *et al.*, 2017).

Free radicals and reactive oxygen species (ROS) are substances that are created spontaneously by metabolic processes in living things which create oxidative stress (Kingne *et al.*, 2018). Moreover, NPs exhibit potent antioxidant properties. By giving up an electron or hydrogen, antioxidants can safely interact with free radicals to stop the chain reaction and change them into harmless molecules. When electrons are accepted by a purple-coloured solution that is apparent in the DPPH test, the solution changes colour to a yellow or discoloured one (Breijyeh *et al.*, 2020). Metal ions, metal complexes, and quaternary ammonium compounds are examples of commercially used antibacterial agents. These substances are said to have numerous drawbacks, such as antibiotic resistance and high expense. Metal oxide nanocomposites have strong antibacterial qualities that compete with multidrug-resistant organisms such as *E. coli*, *S. aureus*, *P. aeruginosa*, *S. mutans*, *K. pneumoniae*, etc. because of their ease of processing, affordability, and environmental sustainability (Babu *et al.*, 2019).

Codoped metal oxide NCs boost antimicrobial efficacy, which in turn raises the formation of ROS because of the increased frequency of host metal oxide NCs active site reactions. Metal oxide NPs and NCs become promising antibacterial agents by breaking down bacterial cell membranes, which helps them overcome microbial drug resistance (Babu *et al.*, 2019). Therefore, this study focused on using phytochemicals from *Mangifera indica* leaf extract due to its rich bioactive compounds, which can boost the biological activities of NCs and provide environmentally friendly approaches for manufacturing ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs for enhanced antioxidant and antibacterial activities.

1.2. Statement of the problems

The majority of bulk materials' properties are fixed, whereas those of nanostructures are readily modifiable, and more interestingly, doping can alter the initial material's activity (Saeed *et al.*, 2020). Therefore, various groups have described the chemical synthesis of Ni-Ag/ZnO NCs (Sonia *et al.*, 2023). However, because it requires hazardous chemicals as reducing and stabilizing agents, this technology has more drawbacks than environmentally friendly ones and limits its biomedical applications (Haq *et al.*, 2020). This encourages us to manufacture Ni-Ag/ZnO nanocomposites using *Mangifera indica* leaf extract, as there has never been a report of using any plant, including *Mangifera indica* leaf extract, for antioxidant and antibacterial applications. Oxidative stress and microbially caused disease are the leading cause of death worldwide and has been linked to a number of degenerative illnesses, including Parkinson's disease, cardiovascular disease, carcinogenesis, and mutagenesis. To solve these problems, researchers are working to create a variety of chemical substances known as medications. Among these agents, nanoparticles and nanocomposites are showing great efficacy against a variety of diseases. The researcher was concentrated on the antioxidant and antibacterial actions of *Mangifera indica* leaf extract in the fabrication of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs and Ni-Ag/ZnO NCs. ZnO NPs, Ni-ZnO NCs and Ag-ZnO NCs have all been previously documented greenly; however, Ni-Ag/ZnO NCs have not been reported greenly up on the application described above.

Therefore, the present study was planned to answer the following questions: Are *Mangifera indica* leaf extracts rich in constituents used in capping, and is stabilizing agents responsible for ZnO NPs, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO NCs synthesis? What is the antioxidant activity of the synthesized samples compared to standard ascorbic acid against DPPH? What are the synergistic effects of *Mangifera indica* leaf extract-mediated Ag-Ni/ZnO NCs on antioxidant and antibacterial activities?

1.3. Objectives of the study

1.3.1 General objective

The main objective of this study was Green synthesis of Ag-Ni/ZnO NCs using *Mangifera indica* leaf extract for evaluation of its antioxidant and antibacterial activities.

1.3.2. Specific objectives

- To screen phytochemicals present in *Mangifera indica* leaf extract.
- To Green synthesize ZnO NPs, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO NCs using *Mangifera indica* leaf.
- To characterize the synthesized nanoparticles and nanocomposites using UV-Vis, XRD, and FT-IR spectroscopy.
- To evaluate the antioxidant using DPPH and antibacterial activities using Disc diffusion method of the synthesized ZnO NPs, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO NCs.

1.4 The Significance of the study

The synthesis of nanomaterials through plants is a superior technique due to its slower kinetics, improved manipulation, and stability. *Mangifera indica*, with its rich phytochemical makeup, has intrinsic antioxidant, antibacterial, antifungal, anti-inflammatory, and anticancer capabilities, making it a great choice for controlled nanoparticle manufacturing. This research investigates the co-doping effects of nickel and silver on ZnO nanoparticles using *Mangifera indica* leaf as a bio-reducing agent. This unique approach aims to close the research gap and establish *Mangifera indica* leaf as an important in environmentally friendly synthesis of Ag-Ni/ZnO nanoparticles, focusing on the antioxidant and antibacterial performance of these nanoparticles.

We also guarantee a thorough examination and comparative assessment of the optical, morphological, and structural aspects of the synthesized NPs. Our study makes a significant contribution to the advancement of sustainable nanotechnology by focusing on co-doping effects, green synthesis, and the use of *Mangifera indica* leaf as a bio-reducing agent. The goal of the research was to examine the antioxidant and antibacterial properties of undoped, monodoped, and Ni-Ag/ZnO NCs. To this end, *Mangifera indica* leaf plant extract was used, which was inexpensive, non-toxic, and easily sourced from the surrounding area. In a variety of industrial applications and fields, this work resolves the side effects of chemical synthesis, and Ni²⁺-Ag⁺/ZnO codopant would enhance the antioxidant and antibacterial activities of ZnO NPs. Additionally, codoping of ZnO NCs is expected to improve the original substances' properties by altering their crystal structures, morphology, size, shape, and surface area to volume ratios, which boosts their biomedical applications (Faye *et al.*, 2022).

Therefore, the synthesised ZnO NPs, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO NCs using *M. Indica* leaf extracts may provide the following significance.

- The synthesized ZnO NPs, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO NCs may be potential inputs for developing a green synthesis method.
- The synthesized ZnO NPs, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO NCs using *Mangifera indica* leaf extract would serve as the stepping stone for the next research development by other researchers.
- The *Mangifera indica* leaf extract mediated synthesized ZnO NPs, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO NCs can be used to enhance the antioxidant ability and eliminate free radicals due to their synergetic effect.
- The *Mangifera indica* leaf extract mediated synthesized ZnO NPs, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO NCs can be used to enhance the antibacterial activity.

2. LITERATURE REVIEW

2.1. Metal oxide Nanoparticles

Nanoparticles, defined as materials with a size of one to one hundred nm in at least one dimension, have significant interest because of their unique and intriguing characteristics and wide range of uses compared to their bulk counterparts (Jeba & Amaladhas, 2018). Because of their exceptional qualities and potential uses in technology, nanostructured transition metal oxides are one of the many forms of nanomaterials that need particular attention (Ash *et al.*, 2018). Thus, rather than being limited to a particular field, nanoscience and nanotechnology cover a wide range of scientific fields, including chemistry, engineering, physics, and medicine (Xavier *et al.*, 2019). Different techniques are used to produce and characterise these compounds. Based on their size, chemical makeup, and place of origin, nanoparticles can be categorised (Claudel *et al.*, 2020). For a variety of basic scientific and technological purposes, as well as to open the door to new classes of functional materials with distinctive characteristics and notable uses, nanostructured metal oxides have been widely identified. The synthesis of nanoscale crystalline metal oxides has garnered significant attention in recent times due to their large surface areas, unique adsorptive qualities, surface defects, fast diffusivities, conductivity, ionic structure, freezing, melting, and colour within the nanorange (Ash *et al.*, 2018).

ZnO exhibits a direct wide band gap of 3.37 eV, strong thermal conductivity, high electron mobility, and good transparency. Furthermore, at normal temperature, ZnO preferably has a stable hexagonal wurtzite, zinc mix, and rock salt structure, with each oxygen ion being encircled by a tetrahedral zinc ion (Ebrahimi *et al.*, 2012). Zinc oxide (ZnO) has garnered significant interest because of its benign properties, including non-toxicity, biocompatibility, stability, and abundance, and it also inhibits the action of pathogenic microbes when used in small concentrations (Abdullah *et al.*, 2024). Numerous metal oxide nanoparticles with potential industrial uses have been developed, and ZnO is the most widely used of these at the nanoscale due to its remarkable scientific qualities, which are ascribed to its enormous excitonic binding energy and band gap (Chaudhuri *et al.*, 2017).

The production of metal oxide nanoparticles (NPs) has attracted a lot of attention lately due to their exceptional surface-to-volume ratios and distinct physicochemical characteristics. Notably, NPs such as titanium oxide (TiO₂) (Irshadet *et al.*, 2021), zinc oxide (ZnO) (Islam *et al.*, 2022), silver oxide (AgO) (Rita *et al.*, 2020), gold oxide (AuO) (Shahet *et al.*, 2022), iron oxide (Fe₃O₄) and silicon dioxide (SiO₂) (Sharf-Eldin *et al.*, 2023), etc. have emerged as focal points of research due to their tunable properties and unique characteristics such as great photosensitivity, high thermal stability, and environmental sustainability (Rajeshkumar *et al.*, 2021). ZnO NPs stand out among them because of their wide band gap semiconductor properties, substantial exciton binding energy, and direct energy band gap between 3.1 and 3.3 eV (Jose *et al.*, 2021). Its diverse applications in optoelectronic devices (Suganya *et al.*, 2024), biosensors (Singh *et al.*, 2019), disease diagnosis (Kanagamani *et al.*, 2021), antibacterial agents (Gudkov *et al.*, 2021), and photocatalysis (Saravanakumar *et al.*, 2022) are significant in the current scientific world. Because of their unique qualities, wide bandgap, high surface area, and superior electrical conductivity, zinc oxide nanoparticles are now in great demand in the domains of solar cells and energy storage technologies. ZnO nanoparticles are frequently used in optoelectronic devices, such as laser devices, optical waveguides, photovoltaic devices, light-emitting diodes, and biomedical imaging devices (Das *et al.*, 2021). Additionally, they have functionalized as protein detection, enzyme biosensors, immunoassays, and DNA biosensors and are known to facilitate label-free biosensing (Hatami *et al.*, 2020). Functionalised ZnO NPs can target particular cancer cells, making these nanoparticles valuable in imaging techniques including fluorescence imaging and photoacoustic imaging. They can also be used in cancer diagnosis and imaging (Ibraheem *et al.*, 2022). Optical or electrical characteristics of ZnO NPs can be used to track the release of medicinal drugs in real-time by adding them to drug carriers (Wiesmann *et al.*, 2020). Zinc oxide nanoparticles (ZnO NPs) are superior to other NPs due to their abundance in nature, chemical stability, and low cost of manufacture. When compared to materials like TiO₂, ZnO NPs demonstrate greater optical, electrical, mechanical, and antibacterial activity (El-Morsy *et al.*, 2023). ZnO is more advantageous economically, as its manufacturing costs are almost 75% cheaper than those of TiO₂ and Al₂O₃ (Ong *et al.*, 2018). ZnO NPs also show minimal toxicity to human cells, which makes them a good option for a number of biological uses, such as medicine delivery and nano-dentistry (Rashid *et al.*, 2024).

Zinc Oxide (ZnO) has garnered attention among other metal oxides because of its distinct biological, physical, and chemical characteristics (Mohammadi *et al.*, 2018). Biomaterials derived from plants have been used to synthesize several nanomaterials that are nontoxic, cost-effective, environmentally friendly, and efficient. Plant-derived phytochemicals have been utilized as reducing and stabilizing agents in the synthesis of metal oxide nanoparticles, while extracts from *Aloe vera*, *Calotropis procera*, *Trifolium pratense* flower, and *Citrus aurantifolia* have been employed to manufacture ZnO nanoparticles. For the synthesis of Fe, Ag, and Ag-doped ZnO NPs, respectively, reducing and stabilizing agents have been employed in the extracts of leaves from *Amaranthus spinosus*, *Prunus cerasifera*, and *Cannabis sativa* (Faye *et al.*, 2022). ZnO nanoparticles wrapped around *Mangifera indica* seeds for antibacterial and antioxidant applications (Rajeshkumar *et al.*, 2023). ZnO nanoparticles were synthesized using *Mangifera indica* leaves for antioxidant and cytotoxic properties in lung cancer (A549) cells (Rajeshkumar *et al.*, 2018). ZnO nanoparticles synthesised using extracts from *Allium sativum* for invitro antioxidant applications (Benshiga *et al.*, 2024). ZnO nanoparticles were synthesized using green *Ephedra alata* leaf extract for phytochemical analysis, antioxidant activity, and antibacterial application (Zaater *et al.*, 2024). Bio-synthesized ZnO nanoparticles derived from *espresso-spent* coffee grounds for their photocatalytic (tartrazine), antioxidant, and antibacterial potential (Ostovar *et al.*, 2023). The cytotoxic and antioxidant effects of ZnO nanoparticles made from *Mangifera indica* leaves were tested on lung cancer cells. ZnO nanoparticles' antioxidant activity was examined using the DPPH method, and it was found that ZnO's antioxidant activity was similar to ascorbic acid's (Khan *et al.*, 2023). Utilizing *Punica granatum L.* waste peel extract, ZnO nanoparticles were created for the antioxidants application (Meshkatsadat *et al.*, 2023). Because of their size and structure, nanoparticles have significant antibacterial and antioxidant characteristics, and the most prevalent method for radical scavenging activity is the generation of reactive oxygen species (Mandal *et al.*, 2022).

2.2. Monodoped metal Oxide Nanocomposites

Transition metal-doped ZnO nanostructures have been the subject of much research in recent decades. Silver nanoparticles (Ag NPs) in particular stand out among metal nanoparticles and their bulk counterparts due to their remarkable features and high activity potential, which has resulted in significant advancements in a number of fields, including water treatment, biosensors, agriculture, and pharmaceuticals (Ratan *et al.*, 2020). Ag nanoparticles can be doped into semiconductor-based heterostructures, like ZnO, to make them multifunctional, and they show remarkable resistance to agglomeration, maintaining effective utilization (Jomehzadeh *et al.*, 2021). As the Ag is bonded in the ZnO structure, the agglomeration and particle size are regulated, lowering self-inter-particle attraction (Makauki *et al.*, 2023). Therefore, dopant can be added to ZnO NPs to adjust their optoelectrical properties; however, the dopant must have both n-type and p-type states. Because of its low orbital energy, large ionic size, and simple solubility, silver is a crucial p-type dopant in this competition. Ag's 4d and O's 2p orbitals overlap to produce an impurity band, which further moves Fermi levels in the direction of the valence band and reduces the energy gap (K Sali *et al.*, 2021).

Many studies have recently concentrated on producing a p-type ZnO by adding dopants, such as As, Ag, Sb, and Ag, which is more common for a variety of applications (Kumar *et al.*, 2019). Phytoconstituents from *P. cerasifera* fruit were used to dope Ag nanoparticles into ZnO nanoparticles in a simple and biomimetic manner (Ahmad *et al.*, 2018). An increasing number of cutting-edge uses for Ag-ZnO NCs have been discovered, such as field-effect transistors, field emission arrays, ultraviolet lasers, light-emitting diodes, sensors, biosensors, catalysts, energy storage, and solar cells (Phoohinkong *et al.*, 2017). In recent years, ZnO has been the subject of interest for transition metal doping as a possible means of modifying its optical and electrical properties. Under controlled circumstances, transition metal ion doping into the ZnO lattice has proven to be a versatile method of enhancing performance for optoelectronics, biomedicine, and electronics applications (Rashid *et al.*, 2024). ZnO nanoparticles' characteristics have been shown to be significantly altered by doping with metal elements like Ni, Co, Sn, Al, Ag, Cu, Sr, Mg, Sb, and Mn, creating opportunities for more advanced applications (Rashid *et al.*, 2024).

Utilization of *Mangifera indica* leaves for Ag NPs produce 20 nm particle sizes and a spherical, triangular, hexagonal form (Gnanasangeetha *et al.*, 2020). Biosynthesized Co-ZnO NCs with *Annona muricata* leaf extract for photocatalytic (MB), cytotoxicity, antioxidant, and antibacterial applications were reported (Vindhya *et al.*, 2023). An environmentally friendly approach for antibacterial, antioxidant, and anti-inflammatory activities using Cu-ZnO-based polymer nanocomposite (Al-Rajhi *et al.*, 2022). Co and Co₃O₄ nanoparticles that are produced sustainably for use in biomedical applications (antibacterial, antioxidant, antifungal, cytotoxic, antileishmanial, larvicidal, and hemolytic properties) (Waris *et al.*, 2021). Ag-ZnO nanoparticles produced using *Morinda citrifolia* fresh leaf extract and their anti-oxidant properties (Shreema *et al.*, 2021). Ag-ZnO nanocomposites with antibacterial, antifungal, antioxidant, and photocatalytic properties were synthesized utilizing *Trigonella foenum-graecum* leaf extract (Noohpisheh *et al.*, 2020).

Doping metals into ZnO NPs is receiving greater attention due to its improved properties and applications (Okeke *et al.*, 2022). The synthesis of ZnO and Ni-ZnO NCs was done using *Euphorbia abyssinica* plant extract for antimicrobial activities (Faye *et al.*, 2022). Ni-ZnO NCs synthesized by a low-temperature wet chemical method and their enhanced field emission properties were observed (Kumar *et al.*, 2023); Ni-ZnO NCs for optoelectronic applications (Mohamed *et al.*, 2021); and Ni-ZnO NCs physicochemical and electrochemical performance (Abinaya *et al.*, 2017). Ni-ZnO NCs synthesized by the sol-gel technique for antibacterial applications (Vijayaraj *et al.*, 2023), zinc oxide, Ni-ZnO NCs poly (O-toluidine) nanocomposite using chemical oxidative polymerization for different characterization techniques (Tamilselvi *et al.*, 2019), Ni-ZnO NCs synthesized via the sol-gel method as antibacterial agents (Alghamdi *et al.*, 2022), Ni-ZnO NCs for structure and optical properties study (Eppakayala *et al.*, 2020), and Ni-ZnO NCs synthesized by a simple co-precipitation technique for near-infrared light-emitting diodes (Elhamdi *et al.*, 2022) were previously reported. ZnO and Cu-ZnO nanocomposite were synthesized from leaf extracts of different plants like *Abutilon indicum*, *Clerodendrum infortunatum*, and *Clerodendrum inerme* for biological activities (antioxidant, antibacterial, antifungal, and anticancer) and photocatalytic application (Khan *et al.*, 2018).

La-ZnO nanocomposites were synthesized by the sonochemical method for their cytotoxicity and antioxidant capacity (Mejía-Méndez *et al.*, 2024). SnO₂ nanoparticles synthesized greenly for antibacterial, antioxidant, cytotoxicity, gas sensor, and catalytic applications (Gebreslassie *et al.*, 2021). The doping of transition metal ions into ZnO nanoparticles has altered their optical and electrical characteristics. This alteration is explained by the exchange interaction between the transition metal ions of d electrons and s and p electrons of the ZnO NPs host. Cr-ZnO, Mn-ZnO and Cr-Mn/ZnO nanocomposites were synthesized by the coprecipitation method (Dhivya *et al.*, 2019).

2.3. Codoped metal Oxide Nanocomposites

Co-doping ZnO NPs with various materials has significant interest in enhancing the efficiency and sustainability of ZnO NPs. Studies have shown that co-doping ZnO nanoparticles with Mn & Co (Rashid *et al.*, 2024), Fe & Co (Luo *et al.*, 2024), and Ni & Co (Ali *et al.*, 2018) exhibit better antimicrobial, photocatalytic, and ferromagnetic properties compared to pure and mono-doped ZnO NPs. Co-doping with Ni & Cu (Ghaznavi *et al.*, 2025), Y & Cu (Mezni *et al.*, 2020), and Al & Cu (Tariq *et al.*, 2022) shows higher dielectric constants, ferromagnetism, and better optical properties suitable for optoelectronic device development. On the other hand, co-doping ZnO NCs with Al & Ag (Sridhar *et al.*, 2023), Ni & Ag (Ullah *et al.*, 2023), and Y & Ag (Ullah *et al.*, 2023) has been observed to have better electrical, optical, and photocatalytic properties. When metallic dopants including Al, Co, Mg, and Mn, among others, are successfully doped with ZnO NCs, ZnO exhibits room temperature ferromagnetism (RTFM), according to earlier research. Using extracts from *G. sylvestre* leaves, the La-Ce/ZnO NCs were created using a green approach and their structural, optical, and antibacterial qualities were examined (Karthikeyan *et al.*, 2018). Cu-Ni/ZnO nanocomposite synthesized via *Dodonaea viscosa* leaf extract for antibacterial and antioxidant applications (Khan *et al.*, 2023). Solar light-driven Ag-Ni/ZnO photocatalysts were synthesized by co-precipitation and sonochemical methods for environmental remediation (Sonia *et al.*, 2023). Ag-Ni/ZnO NCs synthesized by the coprecipitation technique for photocatalytic degradation of Methylene blue (MB) dye (Jeyachitra *et al.*, 2022) and Ag-Ni/ZnO nanomaterials synthesized via the sol-gel method for catalytic degradation of methyl orange (Azfar *et al.*, 2020).

Table 1. Summary of plant-mediated metal-metal codoped metal oxide nanocomposites with their applications.

Codoped metal oxide NCs	Plant used	Applications	Reference
CuO-GO-Ag	<i>Fagonia arabica</i> leaves	Anti-inflammatory and antioxidant activity	Shahid <i>et al.</i> , 2022
Ag-Nd/TiO ₂	<i>Starch</i>	Photocatalytic activity	Bordbar <i>et al.</i> , 2024
Ag-Mo/CuO	<i>Azadirachta indica</i> leaf	Photocatalytic and antimicrobial activities	Rajendaran <i>et al.</i> , 2019
Ni-Co/SnO ₂	<i>Psidium guajava</i> L.	Antibacterial, anticancer and antifungal	Elderderly <i>et al.</i> , 2023
Mn-Cu/ZnO	<i>Corn silk</i> (<i>Stigma maydis</i>)	Antimicrobial and photocatalytic	Wali <i>et al.</i> , 2022
La-Ce/ZnO	<i>Gymnema sylvestre</i> leaves	Antibacterial Properties	Karthikeyan <i>et al.</i> , 2018
MnO-GO-Ag	<i>Fagonia arabica</i> leaves	Anti-inflammatory	Mansoor <i>et al.</i> , 2022
Fe-Ni/SnO ₂	<i>Azadirachta indica</i> Leaf	Antibacterial activities	Aloufi <i>et al.</i> , 2023
Ag-Au/ZnO	<i>Justicia adhatoda</i> leaves	Antibacterial and Anticancer activities	Pandiyan <i>et al.</i> , 2019
Ag-Cu/ZnO	<i>Aloe vera</i>	Antibacterial activities	Rashid <i>et al.</i> , 2024
Cu-Ni-Zn	<i>Dodonaea viscosa</i> leaves	Antioxidant and antibacterial activities	Khan <i>et al.</i> , 2023

2.4. Strategies of Nanoparticles and nanocomposite Synthesis

Several synthesis methods, such as electrodeposition, hydrothermal, spray pyrolysis, combustion, sol-gel, precipitation, and biosynthesis, have been used to produce nanostructured ZnO NPs (Rashid *et al.*, 2024). Green synthesis, which uses eco-friendly ingredients like microbes or plant extracts, is better since it is more environmentally friendly. This approach limits the use of dangerous chemicals, uses less energy, creates non-toxic materials, and complies with green chemistry guidelines (Rashid *et al.*, 2024).

Several types of plants, such as *Azadirachta indica* (Haque *et al.*, 2020), *Mimosa Pudica*, *Moringa oleifera* (Verma *et al.*,2020), *Calotropis procera* (Gawade *et al.*,2017), *Agathosma betulina* (Anhorn *et al.*,2018); lemon juice, orange fruit peel (Thi *et al.*,2020), *Calotropis procera* (Gawade *et al.*,2017), *Hibiscus sabdariffa* (Soto-Robles *et al.*,2019), *Syzygium Cumini* (Sadiq *et al.*,2021), *Carica papaya* (Rathnasamy *et al.*,2017) and *Aloe barbadensis miller* (Nagaraj *et al.*,2021) have been studied extensively for their potential as a green synthesis method to improve the efficiency and sustainability of optoelectronics, biomedical, energy storage, and catalysis applications. Generally, there are two methods for creating NPs: the top-down method and the bottom-up method.

i) Top-Down Approach for NPs Synthesis. Top-down entails disassembling bigger molecules into smaller ones and turning them into nanoparticles. Top-down procedures include processes like as sputtering, chemical etching, laser ablation, mechanical milling, and electro-explosion. This process consists of a collection of artificial technologies that create NPs by extracting certain components from a substrate made of bulk materials. Parts can be extracted from bulk materials using a variety of techniques, such as mechanical, chemical, and electrochemical approaches. The material of the bulk substrate and the most desired NP sizes determine which approach is used. However, complete control over particle size is not possible with this technology. To achieve nanosized regions, the top-down approach is expanded, and mechanical removal systems are coupled with chemical and electrochemical methods (Khan *et al.*, 2019).

ii) Bottom-Up Approach for NPs Synthesis. This method involves stacking elements on top of a base substrate while keeping good control over the molecular structure. It uses a collection of synthetic technologies to create larger and more complicated systems. Strong adhesive forces between the surface layer and base substrate are one of the fundamental prerequisites for this production method. Surfactants are added to this mixture to improve adhesion between the surface layer and base substrate (Khan *et al.*, 2019).

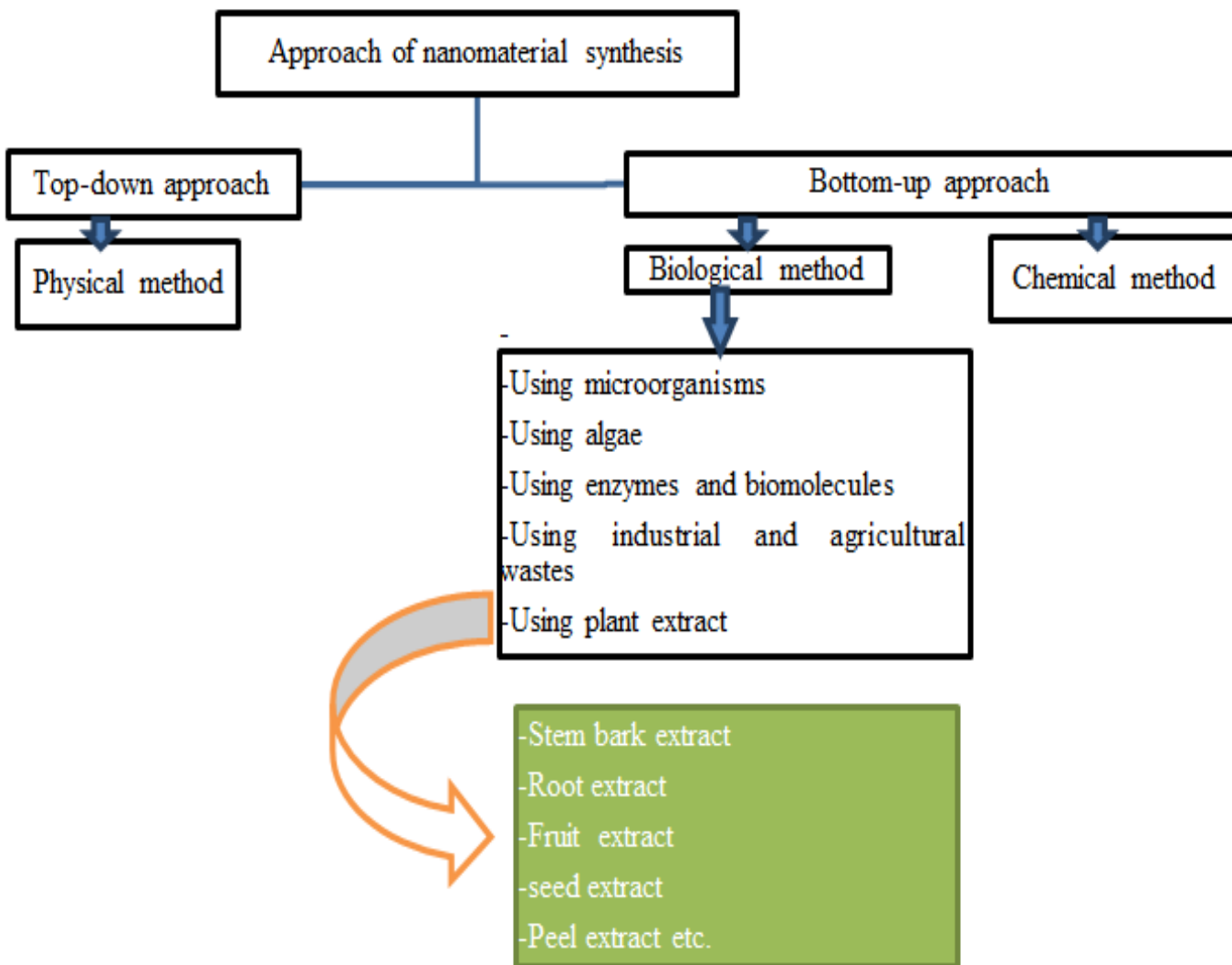


Figure 1. Various methods for the synthesis of NCs.

2.5. Factor affecting green synthesis of nanomaterials

For nanoparticles and nanocomposites to have the right size, stability, and quality, optimization is necessary. According to the guidelines set forth in earlier reports, a number of factors that control the experimental conditions that are appropriate for the synthesis of nanoparticles will be adjusted by changing one at a time while holding the other constant in order to achieve the best results.

2.5.1. Effect of Concentration of Metal Precursors

The end products of NP synthesis are also influenced by changes in the amounts of metal salts or plant material. The peak from the synthesized AgNPs, which were made from banana peel extract, grew increasingly pronounced as the concentration of AgNO₃ increased from 0.25 to 2.0 mM (Saeed *et al.*, 2020). The highest peak was achieved at a concentration of 1.75 mM. Furthermore, the Ag NPs had a bigger average size of 350 nm at increasing concentrations. The synthesized Ag and Au NPs from various metal concentrations (1, 2, and 3 mM) were shown to have decreased absorbance and sluggish NCs formation at the lowest concentration (Alex *et al.*, 2020).

2.5.2. Effect of volume of plant extracts

The types of biomolecules present in plant extracts and the volume employed are the primary determinants of the production of NPs utilizing plant extracts (Devatha, C. P. *et al.*, 2018). The volume of plant extracts used determines how long it takes to create nanoparticles. A recent study showed that the larger the amount of extract used, the faster the rate of synthesis is because more chemical components in the solution bond with the precursor to affect speedy bio-reduction and stability of nanoparticles. To obtain the ideal circumstances for the environmentally friendly synthesis of NPs, the volume of plant extract and the concentration of metal precursor used must match. The formation of NPs is significantly influenced by the amount of extract used for synthesis. The physical characteristics and biological activities of nanoparticles are significantly influenced by the type and volume of extract utilized during synthesis. Ag NPs were synthesized using varying quantities of *Citrus sinensis* peel extract. At the volume range of 2.5 - 7.5 mL, the SPR band intensity rose somewhat as the extract volume increased because more NPs were formed. However, because large-sized NPs grow at larger concentrations, the SPR band's intensity reduces (Naseem *et al.*, 2020).

2.5.3. Effect of Reaction Temperature

Temperature has been found to be one of the most significant variables affecting the production of metallic oxide nanoparticles. Temperature is one of the most important factors that significantly influence the size, shape, stability, and yield of the NPs made using a green approach. When employing plant extracts to create metallic oxide nanoparticles, a temperature range of 25 to 100 °C is advised (Jeevanandam *et al.*, 2022).

However, the synthesis of NPs at room temperature or lower is more selective since some secondary metabolites in plant extracts are volatile. The morphological identity of nanoparticles is influenced by the temperature of the reaction solution. Results from the green synthesis of metallic oxides using plant extracts have shown rapid and complete synthesis at higher temperatures. Higher temperatures, however, have been shown to degrade nanoparticle formation because they inactivate the biomolecules responsible for ion precursor reduction (Chugh *et al.*, 2021).

2.5.4 Effect of pH

One significant aspect influencing the production of NPs is pH. Raising the pH in the reaction mixture causes a decrease in the overall particle size and an increase in small-sized NPs. The size and texture of the produced nanomaterial is affected by the pH of the solution media. Due to the ionization of the tannins and phenolic chemicals in the plant extract, a further increase in pH caused the reaction rate to drop. Also, adjustments in the pH value have been employed in controlling the form and size of the produced nanoparticles as applied by (Arya *et al.*, 2021).

2.6. Plant-mediated synthesis of nanoparticles and nanocomposites

Metal oxide nanocomposite materials (NCs) are frequently synthesized from plants because of their accessibility, stability, safety, and lower levels of phytochemicals. Plants have long demonstrated the ability to take in, store, and release inorganic metallic ions from their surroundings. It is now widely known that a variety of organic substances found in plant tissues can function as efficient biological factors to greatly lower environmentally friendly contamination, recover metals from industrial waste, and expand the use of nanocomposites in biomedicine. Utilizing biological systems such as bacteria, fungi, yeast, plants, and naturally occurring tiny molecules like vitamins, proteins, peptides, and reducing sugars, bionanotechnology creates NPs. It also has the added benefit of stabilizing the NPs as secondary metabolites of plants (Khan *et al.*, 2019). Secondary metabolites including flavonoids, terpenoids, polysaccharides, tannins, phenols, and saponins all have significant functions. The main mechanism for this process is plant-assisted reduction, and the choice of suitable capping moieties has a significant impact on the stability of the nanocomposite (Basnet *et al.*, 2018). The surface chemistry and size distribution of nanocomposites are altered following their capping with biocompatible surfactants (Aisida *et al.*, 2019).

Numerous bioactive substances present in plants function as stabilizing and reducing agents, which can facilitate the creation of metal oxide nanocomposite (Begum *et al.*, 2022). The secondary metabolites of plants are accountable for the reduction of metal ions into metal atoms. The phytochemicals in leaves, like phenolic compounds, flavonoids, phenolic acids, alkaloids, and tannin content, help in the reduction of metal ions to metal nanoparticles and act as good capping and stabilizing agents by adsorbing onto the surface of NPs, which forms a protective layer around NPs, preventing them from agglomerating or aggregating (Kaur *et al.*, 2024). The metal salts like nitrates, chlorides, oxides, and sulfates have high reduction potential due to the attachment of metal with the chloride, oxide, and sulfide parts and their tendency to donate electrons (Chokkareddy & Redhi, 2018). So, metals in their ionic form can easily get detached from their anionic part and get reduced into stable form by using plant extract to form NPs.

2.7. *Mangifera Indica* plant

Mango (*Mangifera indica*-family Anacardiaceae) is a tropical plant with a unique nutritional and phytochemical makeup, according to Ayurveda and indigenous medicinal traditions (Burton-Freeman *et al.*, 2017). *Mangifera indica*, a significant tree with a number of therapeutic uses, is a member of the Anacardiaceae family and is popularly known as Mango (Table 2). One of its bioactive components, mangiferin, has been shown to have strong antioxidant properties (Yehia *et al.*, 2023). Furthermore, it has been discovered that extracts from its leaves and stem bark have strong anti-inflammatory, anti-cancer, anti-fungal, anti-microbial, and anti-malarial properties (Batoool *et al.*, 2018). Mangiferin and other phytochemicals such as phenolic acids, benzophenones, and other antioxidants like flavonoids, ascorbic acid, carotenoids, and tocopherols are abundant in *Mangifera indica* leaves, which are used in the green synthesis of Ag-Ni/ZnO NCs (Kumar *et al.*, 2021). *Mangifera indica* leaves are abundant in phytochemicals, which are essential for promoting health, preventing disease, and making drugs (Dhivya *et al.*, 2019). Phenolic acid, tannins, and flavonoids are three forms of polyphenols that exhibit both anti-mutagenic and anti-carcinogenic properties. According to recent findings, *Mangifera indica* leaf extract has the potential to be used in the creation of nanostructures (Panwar *et al.*, 2022). The phytochemicals found in leaves, such as tannin content, diterpenes, phenolic acids, and flavonoids, aid in the reduction of metal ions to metal nanoparticles and function well as capping and stabilizing agents (Kaur *et al.*, 2024).

The formation of zinc oxide nanoparticles (ZnO NPs) is supported by the usage of extensive phytochemical content found in plants, such as flavonoids, saponins, carotenoids, alkaloids, and polyphenols. *Mangifera indica* leaves, which are abundant in phenolic acids, xanthenes, benzophenones, tannins, terpenoids, and flavonoids, have been used in the production of ZnO NPs (Kumar *et al.*, 2021). While ZnO NPs synthesized using mango leaf extract (Sierra *et al.*, 218) and mango seeds (Rajeshkumar *et al.*, 2023) are recognized for medical applications and their photocatalysis purposes have been reported. *Mangifera indica* leaf extract were used to synthesize silver nanoparticles and observed that the resulting nanostructures exhibited good photocatalytic activity and synthesised carbon quantum dots using *Mangifera indica* leaf extract for application in bioimaging (Kumawat *et al.*, 2017). *Mangifera indica* leaves were utilized to make Au NPs have a spherical form and a particle size range of 17 - 20 nm (Khan *et al.*, 2022). According to literature, Mango leaf extract has been utilised for the fabrication of silver nanoparticles (Simhan *et al.*, 2017), iron as well as iron oxide nanoparticles (Saha *et al.*, 2023), gold nanoparticles (Sahadewo *et al.*, 2023), and zinc oxide nanoparticles (Akhtar *et al.*, 2023). Several studies have mentioned the utilisation of mango peels for the synthesis of TiO₂, Ag-TiO₂, and Se-ZnO Nanocomposites using *Mangifera indica* leaf extract for photodegradation and antibacterial applications (Dhivya *et al.*, 2019); Er-ZnO Nanocomposites synthesised using *Mangifera indica* gum for photodegradation of Methylene Blue, Eosin, and Ibuprofen were reported (Silva *et al.*, 2024); but none, to the best of our knowledge, have utilised *Mangifera indica* leaves and other plant extracts for green synthesis of Ag-Ni/ZnO NCs except its monodoped to ZnO NCs.

Table 2. Taxonomical Classification of *Mangifera indica* plant

S/N	Scientific Category	Classification
1	Kingdom	Plantae
2	Phylum(Division)	Magnoliophyta
3	Class	Magnoliopsida
4	Order	Sapindales
5	Family	Anacardiaceae
6	Genus	Mangifera
7	Species	Mangifera Indica



Figure 2. Image of *Mangifera indica* plant.

2.7.1. *Mangifera indica* Leaf Constituents

A feature of the climate Mangiferin, selected anthocyanidins such as delphinidin, peonidin, cyanidin, leucoanthocyanins, catechin, Gallic, and tannins have all been shown to be present in *Mangifera indica* leaves. Depending on the variety and region of origin, the mangiferin content of *Mangifera indica* leaves can range from roughly 2-15%, while extracts of mango leaves can contain up to 60% of mangiferin. Mangiferin, Flavonoid, Quercetin, gallic acid, and xanthonoids are among the polyphenolic antioxidants found in high concentrations in *Mangifera indica* leaves (Kumar *et al.*, 2021).

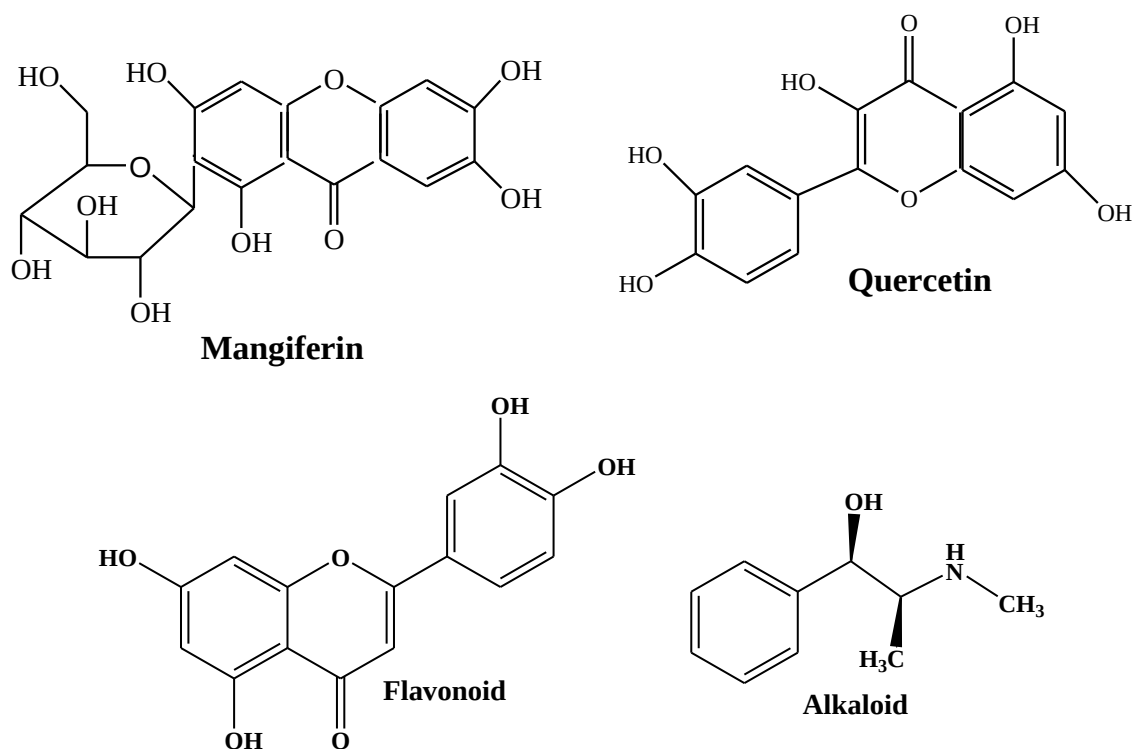


Figure 3. Structure of Phenolic compounds found in *Mangifera indica* plant extract.

Beyond its well-known fruits, the mango tree has numerous traditional therapeutic uses. *Mangifera indica* leaves have the potential to include the following minerals: iron, sodium, calcium, magnesium, phosphorus, potassium, nitrogen, and vitamins A, B, C, and E. Proteins are a significant biomolecule found in *Mangifera indica* leaves. Traditional medicine has used *Mangifera indica* leaf extracts to treat a variety of illnesses, including diabetes, syphilis, bronchitis, diarrhea, asthma, renal disease, scabies, respiratory issues, and urinary difficulties (Al-Snafi *et al.*, 2021). Mangiferin is the most biologically active component of *Mangifera indica* leaves, with phenolic acids, benzophenones, and other antioxidants such as flavonoids, carotenoids, isoquercetin, quercetin, ascorbic acid, and tocopherols following in order of importance. The majority of the biological activity of the *Mangifera indica* leaf extract is mostly attributed to mangiferin (Figure 3). Because of *Mangifera indica* leaves are known to have a variety of phytochemical, biological, and pharmacological qualities, including antimicrobial, antioxidant, antidiabetic, antitumor, and immunomodulatory actions, there is a lot of potential for mango leaf value (Kumar *et al.*, 2021).

2.7.2. Antioxidant and Antibacterial Benefits of *Mangifera Indica* Leaf Extract

Mangifera indica leaves, bark, seeds, flowers, and both raw and mature fruits have been suggested in previous research to have a variety of therapeutic, pharmacological, and physiological effects (Imran *et al.*, 2017). *Mangifera indica* leaves and fruits have been shown in studies to have a variety of health benefits, including hepatoprotective, gastroprotective, immunomodulatory, anti-tumor, wound healing, anti-pyretic, anti-bacterial, anti-spasmodic, anti-carcinogenic, anti-viral, anti-microbial, and anti-dysentery properties (Imran *et al.*, 2017). The flavonoids, terpenes, and phenols present in *Mangifera indica* leaves are responsible for its antioxidant activity. Strong antioxidants and phenolic substances can stop free radicals from causing tissue damage (Rupasinghe *et al.*, 2017). The notable phenol and flavonoid concentration of extracts could be the cause of their antioxidant qualities (Maharaj *et al.*, 2022). Because of its high concentration of antioxidants, primarily phenols (flavonoids, xanthenes, and phenolic acids), mango leaf has been shown to have potential pharmacological benefits (Al-Snafi *et al.*, 2021). According to studies, n-Hexane, acetone, methanol, and other solvents are more harmful to animal models than herbal extracts that are aqueous or ethanolic (Olasehinde *et al.*, 2018).

Plant leaf extracts exhibited antimicrobial properties owing to their rich phytochemical composition (Dash *et al.*, 2022). Leaf extract from *Mangifera indica* has demonstrated biological qualities that make it useful for treating a number of illnesses. The presence of steroids, tannins, flavonoids, and phenols in *Mangifera indica* leaves is responsible for their antibacterial properties. These substances are effective antifungal and antibacterial agents that could contribute to the antibacterial qualities of *Mangifera indica* plant leaves (Maharaj *et al.*, 2022).

2.8. Characterization technique for nanoparticles and nanocomposites.

2.8.1. UV-Visible spectroscopy

The absorption in the visible ranges is directly impacted by the colour of the substances involved. These electromagnetic spectrum bands are where molecules go through electronic transformations (Kalaichelvi *et al.*, 2017). The foundation of UV-Vis spectroscopy is the idea that different spectra are produced when chemical substances absorb ultraviolet or visible light. Utilising UV-visible spectroscopy, the band gap energy of the produced nanoparticles was demonstrated. With smaller particles, there is a blue shift (an increase in the band gap) and a change in the absorption edge to a higher energy. Electronic charge transfers and d-d electronic transitions are identified, and their energies are calculated using electronic spectra. Tauc's charts, which show the fluctuation of $(\alpha h\nu)^n$ versus $h\nu$, are used to extrapolate the linear component and determine the precise optical band gap value. This implies that the sample's optical transition is either direct or indirect, and it is used to calculate the optical band gap of the nanoparticles. The band gap energy of the material can be obtained by extrapolating the linear section of the curve to the X-axis on a tauc plot, which has the photon energy ($h\nu$) on the X-axis and a quantity $(\alpha h\nu)^n$ on the Y-axis (Xavier *et al.*, 2019).

2.8.2. Fourier Transformation Infrared (FT-IR) Spectroscopy

An indispensable tool for describing matter's molecular structure is infrared spectroscopy. Over the past ten years, FT-IR spectroscopic analysis, a technique founded on the principle of infrared spectroscopy, has expanded to its field of use to include the examination of objects at the nanoscale. There are many uses for FT-IR spectroscopy, including the examination of complexes and tiny molecules or molecular complexes (Claudel *et al.*, 2020).

Vibrations in molecules are investigated via infrared spectroscopy. The fundamental vibrations of functional groups correlate to characteristic infrared absorption bands that can be linked to them. Using an FT-IR spectrometer, one can examine how atoms in a molecule twist, bend, rotate, and vibrate when exposed to infrared radiation. A portion of the incident radiation is visible at a particular wavelength, and the spectrum can be used to identify the sample's functional groups (Xavier *et al.*, 2019). The investigation of surface chemistry and the identification of potential biomolecules for the capping and stabilisation of nanoparticles can be greatly aided by FT-IR (Nagajyothi *et al.*, 2020). The ranges 4000-400 cm⁻¹ was identified as potential functional groups that may be present in the plant extract and manufactured nanoparticles.

2.8.3. X-ray diffraction (XRD) Powder

The XRD pattern explains the quantitative study of the nanoparticles and was utilised to identify their purity (Xavier *et al.*, 2019). On an X-ray diffractometer, XRD patterns are gathered for phase identification. A cathode ray tube produces these X-rays, which are then collimated to concentrate them, filtered to produce monochromatic radiation, and directed toward the sample. An X-ray incident beam interacts with itself when it exits a crystal due to the atomic planes of the crystal. X-ray diffraction (XRD) is a non-destructive method that may be used to quantify the thickness of thin films and multilayers, identify crystalline phases and orientation, and ascertain the atomic arrangement and structure of nanoparticles (Habtemariam, 2020). Moreover, information on lattice characteristics, crystallite sizes, flaws, and stresses is obtained using the XRD technique. Scherrer's formula (Eq.1) will be used to determine the nanoparticles' average crystallite size.

$$D = \frac{k\lambda}{\beta \cos\theta} \dots\dots\dots (1)$$

Where D is crystal size, K is an empirical constant to 0.9 and λ is the wavelength of the incidence beam ($\lambda=1.5406\text{\AA}$) (Haq *et al.*, 2021). β is the full-width half maximum (FWHM) in radian, and θ is the angular of the peak position.

2.9. Antioxidant and Antibacterial Activities of Nanoparticles and Nanocomposites

2.9.1. Antioxidant activity of nanoparticles and nanocomposites

Free radicals and reactive oxygen species (ROS) are substances that are created spontaneously by metabolic processes in living things. They aid in the body's defense against infections and microbes; thus, they have some positive effects. On the other hand, when they are created in excess, the organism experiences oxidative stress (Kingne *et al.*, 2018). Since oxidative stress has been linked to numerous degenerative disorders, including mutagenesis, cardiovascular disease, carcinogenesis, Parkinson's disease, acquired immunodeficiency syndrome, which is a chronic illness, neurological disorders, and many more, it is now widely recognised to be the leading cause of death worldwide (Djikeng *et al.*, 2017). In addition to having an adverse effect on living things, oxidative stress damages food products by encouraging oxidation reactions in oils, fats, and foods that include lipids, which lowers the nutritional value and organoleptic qualities of those products (Djikeng *et al.*, 2017). It has been demonstrated that a number of biological substances can stop or postpone oxidative damage in foods and living things. These compounds have the ability to give up one hydrogen atom to help stabilize reactive oxygen species, or free radicals, and they are referred to as antioxidants (Djikeng *et al.*, 2017).

In order to prevent or reduce radicals and to produce less aggressive chemical species that are prone to cause tissue damage, antioxidant activity entails intercepting reactive oxygen species (Kumar *et al.*, 2020). Because antioxidants can shield the body from the damaging effects of reactive oxygen species, using them has attracted a lot of attention (Zhang *et al.*, 2021). Antioxidants can also be defined as substances that have the ability to block particular oxidising enzymes, react with oxidising agents before harming other molecules, sequester metal ions, or even repair systems like iron-transporting proteins. In order to prevent or reduce radicals and to produce less aggressive chemical species that are prone to cause tissue damage, antioxidant activity entails intercepting reactive oxygen species (Kumar *et al.*, 2020). Therefore, it is reported that a variety of physiological and biochemical activities in the human body produce free radicals and other reactive oxygen species. Antioxidants can be classified into two categories: natural and synthetic. Moreover, NPs exhibit potent antioxidant properties, and using *S. thea* leaf extract, plant-mediated cobalt oxide nanoparticles (NPs) with cubic morphologies and an average particle size of 20.03 nm were created (Maharaj *et al.*, 2022).

The biogenic cobalt oxide nanoparticles demonstrated an average total antioxidant capacity and total reducing power in addition to excellent radical scavenging potential (Khalil *et al.*, 2020). Ag-ZnO nanocomposites with antibacterial, antifungal, antioxidant, and photocatalytic properties were synthesized utilizing *Trigonella foenum-graecum* leaf extract (Noohpisheh *et al.*, 2020). The antioxidant activity of nanocomposites in vitro has demonstrated their pharmacological relevance. The DPPH assay is a quick, easy, sensitive, and very promising technique for destroying or lowering free radicals in order to examine the antioxidant capacity of nanoparticles (Zare *et al.*, 2019). " IC_{50} " refers to the amount of a substance that may inhibit 50% of free DPPH radicals. A lower IC_{50} suggests that more free radicals can be eliminated (Zare *et al.*, 2019). When antioxidant activity was measured in the current study using DPPH free radicals, the results indicated that, in contrast to ascorbate, the conventional antioxidant, antioxidant activity depended on the amount of synthetic Ag-ZnO nanocomposites at optimal concentrations. Further observations of color changes in the solution were made in relation to the concentration of nanoparticles. Functional groups on the surface of Ag nanoparticles are responsible for their antioxidant qualities (Keshari *et al.*, 2020).

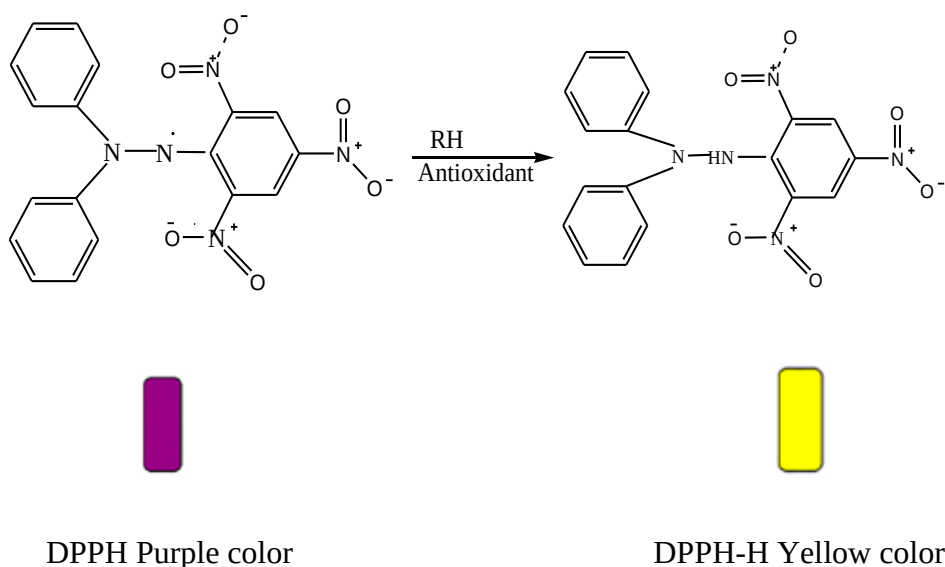
According to studies, Ag incorporated into ZnO nanocomposites has a synergistic impact that increases their antioxidant capacity and removes free radicals (Zare *et al.*, 2019). Ag-ZnO nanocomposites therefore exhibit greater antioxidant activity in terms of DPPH free radical scavenging than ZnO nanoparticles (Noohpisheh *et al.*, 2020). The green-synthesized NPs had good DPPH free radical scavenging activity and showed outstanding results (Hou *et al.*, 2020). Likewise, it has been observed that the DPPH radical scavenging activity of cobalt oxide nanoparticles derived from *Sesbania sesban* extract is lower than that of silver and copper oxide nanoparticles (Ghadi *et al.*, 2018). ZnO NPs and Cu-ZnO NCs for biological (antioxidant, antibacterial, antifungal, and anticancer) and photocatalytic applications were synthesised from leaf extracts of *Abutilon indicum*, *Clerodendrum infortunatum*, and *Clerodendrum inerme*. A higher value of IC_{50} in number indicates that the tested nanoparticles are exhibiting less scavenging activity, and vice versa. It is therefore clear that, in comparison to undoped ZnO nanoparticles, Cu-ZnO nanocomposites produced enhanced DPPH free radical scavenging activity (Khan *et al.*, 2018).

Ag-ZnO nanocomposites were created using fresh *Morinda citrifolia* leaf extract, and DPPH was used as a free radical in the radical scavenging technique to measure the antioxidant activity of the particles. This technique is predicated on the antioxidant scavenging of DPPH, which results in the reduction reaction-induced decolorisation of the DPPH methanol solution. The DPPH methanol solution's hue varies from deep violet to colorless or pale yellow, according to the results. The concentration of Ag-ZnO NCs determines the antioxidant action (Shreema *et al.*, 2021). At maximal concentration, a maximum of around 83% is found for the radical scavenging activity of Ag-ZnO NCs. It has previously been observed that adding Ag to the ZnO lattice improves antioxidant activity and eliminates free radicals because of the synergistic effect (Noohpisheh *et al.*, 2020). Ag-ZnO nanocomposites made from leaf extract can have functional groups that improve their antioxidant capabilities (Shreema *et al.*, 2021). Sulfur and nitrogen coded zinc oxide nanocomposites were synthesized by the sol-gel method for antioxidant properties and antibacterial applications (Yadesa *et al.*, 2024).

Eco-friendly SnO₂ nanoparticles for gas sensors, cytotoxicity, antioxidants, antibacterial, and catalytic applications were reported (Gebreslassie *et al.*, 2021). Numerous researchers used the ability of NPs to quench persistent DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals into nonradical form (DPPH-H) in order to assess the antioxidant activity of NPs. SnO₂ NPs biosynthesized using extracts from *Cleistanthus collinus* leaves were found to have antioxidant action. In another study, the free radical scavenging activity of green synthesized SnO₂ NPs increased in a dose-dependent manner. In contrast to the prepared sample, the annealed sample showed less scavenging activity (Matussin *et al.*, 2020).

There could be a decrease in the NPs' surface area to volume ratio as the annealing temperature rises, which would explain the decline in scavenging activity. The generation of reactive oxygen species is the most prevalent method of radical scavenger (Meshkatsadat *et al.*, 2023). Furthermore, the transfer of electron density between the NPs and the free radical found at nitrogen in DPPH is what gives SnO₂ NPs their antioxidant activity against DPPH. Ag-ZnO NCs produced using *Trigonelle foenum-graecum* aqueous extract showed a comparable outcome (Noohpisheh *et al.*, 2020). When electrons or hydrogen are accepted by a purple-colored solution that is apparent in the DPPH test, the solution changes color to a discolored one (Breijyeh *et al.*, 2020) (Scheme 1). The degree of color change is correlated with the potency and amount of antioxidants that are present (Herrera *et al.*, 2018).

The degree of discoloration is a measure of the effect of free radical scavenging. The measure of the DPPH free radical scavenging activity was the antioxidant-induced drop in absorbance at 517 nm (Manjula *et al.*, 2018). The ability of the nanomaterial to decrease the stable radical DPPH and any other molecule that could contribute hydrogen or an electron to DPPH was used to study its radical scavenging capabilities. Nearly all of the nanoparticles showed a dose-dependent shift in radical scavenging capabilities with increasing concentration, indicating an increase in the DPPH radical scavenging activity (Maharaj *et al.*, 2022). By immobilizing functional groups, the process and modification of inorganic nanocomposites can produce antioxidant activity. To scavenge radicals, DPPH, for instance, can be modified on ZnO nanoparticles (Di *et al.*, 2020). By connecting functional moieties or entrapping bioactivities on the surface of the nanocomposite, it is possible to combine nanoscale particles with antioxidant properties (Ge *et al.*, 2022). For their antioxidant qualities, plant extracts can also be absorbed on the surface of nanoparticulates (Kumar *et al.*, 2021). CuO-GO-Ag nanocomposite's antioxidant potential was assessed utilizing the common ascorbic acid DPPH assay. After synthesizing the sample and standard at different concentrations, a 0.1 mM methanolic DPPH solution was added. At 517 nm, absorption was detected (Shahid *et al.*, 2022).



Scheme 1. Mechanisms of antioxidants activity.

2.9.2. Antibacterial activity of nanoparticles and nanocomposites

Any material, whether natural, semisynthetic, or synthetic, can be used to create antibacterial agents. With little to no harm to the host cell, they can destroy or stop the proliferation of germs one after the other. The Greek words "anti," which means against, "mikros," which means small, and "bios," which means life, were generally combined to form the word "antimicrobial." All microorganisms are susceptible to the effects of antimicrobial compounds, including bacteria (antibacterial), viruses (antiviral), fungus (antifungal), and protozoa (antiprotozoal). Because of their small size, easy permeability through cell membranes, and rapid transport throughout the body, nanoparticles have been widely used as an alternative to conventional medications in recent decades. Scientists are developing these particles as an effective alternative and unique quality of resistance toward an infection (Qamar *et al.*, 2017).

Because of their biocidal, antiadhesive, and transport properties, nanoparticles were utilized to regulate the development of biofilms in the oral cavity. They were also utilized in dental materials and prosthetic devices as topical applied agents. A few specific nanoparticulate systems have also been investigated for their antibacterial properties, including silicon, copper, zinc, silver, and their oxides (Fan *et al.*, 2021). NPs and other conjugates have been mixed with various organic and inorganic substances to enhance the antibacterial response. Ag is a natural antibiotic that has long been recognized for its antibacterial and antioxidant qualities. It can produce reactive oxygen species (ROS) that are effective against a range of bacterial strains. The most well-known inorganic antimicrobials are silver nanoparticles, which are frequently employed in a variety of applications to prevent bacterial growth, fungal infections, inflammations, etc. Compared to Gram-negative bacteria, ZnO NPs were found to be more efficient against Gram-positive bacteria. This resulted from the differing makeup of the bacteria's cell walls; Gram-negative bacteria are more resistant to ZnO NPs than Gram-positive bacteria because they have lipopolysaccharide molecules on the outer membrane layer (Ostovar *et al.*, 2023). However, a number of variables can affect the nanoparticles' biocidal reaction, including flaws in the particles, sample concentration, variations in cell structure, the presence of bioactive chemicals in plant extract, microbial metabolism, etc. (Kumar *et al.*, 2019). Furthermore, because of its rich phytochemical makeup, which includes alkaloids (ephedrine and pseudoephedrine), flavonoids, tannins, polyphenols, and essential oils, leaf extract possesses antibacterial and antioxidant qualities.

While flavonoids and polyphenols function as antioxidants and have antibacterial properties, alkaloids interfere with the microbial cell membrane and metabolic activities. Proteins are precipitated by tannins, which damages microbial cells. These bioactive substances work in concert with NCs to increase the nanocomposites' overall antibacterial and antioxidant effectiveness (Zaater *et al.*, 2024). A number of methods for improved antibacterial action have been explained by the antibacterial action of nanocomposites, including Bacterial cell death is thought to be primarily caused by metal and metal oxide nanoparticles (NPs) disrupting cell membranes, producing reactive oxygen species (ROS), and inhibiting enzymes. Additionally, the bacterial membrane's negative charge interacts with the positive charge of the metal ions in NPs and NCs by electrostatic attraction, rendering them inactive (Pandiyan *et al.*, 2019). Reactive oxygen species (ROS) are produced and the bacterial membrane is damaged by the electrostatic contact, which primarily kills the bacteria. Ag-Au/ZnO NCs enter bacteria, react with thiol or sulfhydryl (-SH) groups on proteins, and deactivate the protein by forming a stable S-metal complex. Increased Ag-Au/ZnO penetrability and inhibition of bacterial metabolism result from protein hydrogen ion loss, which damages the cell membrane. Increased antibacterial activity results from the absorption of visible light energy by small bandgap energy. Accordingly, NCs relied on various toxicological characteristics, including oxygen flaws, nanostick form, tiny particle size, and low bandgap energy (Pandiyan *et al.*, 2019).

3. MATERIALS AND METHODS

3.1. Chemicals and Reagents

All the chemicals and reagents used in this study were analytical grade. Nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 98%), zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 99%), silver nitrate (AgNO_3 , 99.8%), Dimethylsulfoxide ($\text{C}_2\text{H}_6\text{OS}$, 99.9%), Muller Hinton agar media, hand sanitizer, ciprofloxacin ($\text{C}_{17}\text{H}_{18}\text{FN}_3\text{O}_3$, 15 μg), DPPH ($\text{C}_{18}\text{H}_{12}\text{N}_5\text{O}_6$, 99.8%), methanol (CH_3OH , 99.85%), absolute ethanol ($\text{C}_2\text{H}_6\text{O}$, 99.9%), ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$, 99.0%), sodium hydroxide (NaOH , 97%), sulfuric acid (H_2SO_4 , 98%), nitric acid (HNO_3 , 99.9%), Wagner's reagent, ferric chloride (FeCl_3 , 98%), hydrochloric acid (HCl , 36%), and deionized water were used for this research.

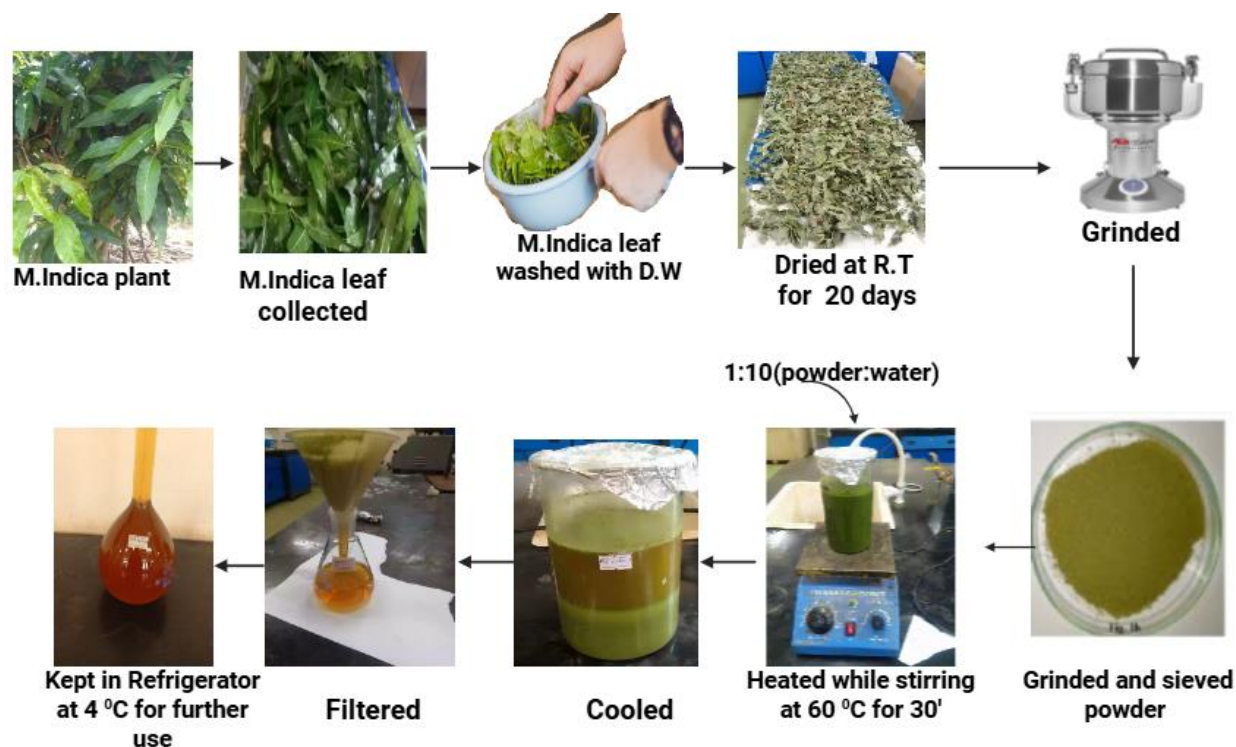
3.2. Instrument, Apparatus, and Equipment

Hot air oven (model 001, India), mortar and pestle, electronic weighing balance (model NWT100001X), hot plate and magnetic bar, whatman No. 1 filter papers, beakers (small and large), test tubes, electrical miller, sieve, volumetric flask, measuring cylinder (different volumes), Erlenmeyer flask, aluminum foil and micropipette were used. The crystalline nature and size of samples to be synthesized were identified with the aid of the X-ray diffractometer (XRD-7000 x-ray diffractometer, Shimadzu, Japan). The FT-IR spectrometer (IS50 ABX.USA) was used to examine the functional groups. Absorption spectra were recorded with the UV-Vis spectrophotometer (MANTI, MT-137V, 1600 microprocessor); biobase and autoclave (automated moist sterilizer, India) were used.

3.3. Collection and Preparation of *Mangifera Indica* Leaf Extract

Mangifera indica leaves were chosen randomly and collected from an open field in Wallaga University's Compound, Guto Gida district, East Wallaga zone, Oromia regional state, Ethiopia, in April 2024. A botanist performed the taxonomic identification and authentication. The collected leaves were cleaned with tap and deionized water, respectively, to remove any dirt or other contaminants, and then allowed to air dry at room temperature for 20 days under shade to remove the moisture content. The dried biomass was then sieved, ground with an electrical miller, and stored in a dry location within a tightly sealed container. 10 g of dry powdered *Mangifera indica* leaf was added to a 500 mL conical flask that held 100 mL of deionized water.

The resultant parts have been boiled for approximately 30 minutes at 60°C while covered with aluminum foil to prevent light exposure. Whatman No. 1 filter paper was used to filter the suspension once it had cooled to room temperature. Lastly, for later use, the brown-green filtrate was put in a glass bottle wrapped in aluminum foil and kept in the refrigerator at 4°C (Firisa *et al.*, 2022). The figurative flow chart for preparing *Mangifera indica* extract was depicted (Scheme 2).



Scheme 2. Extraction of *Mangifera indica* leaves extract flow chart.

3.4. Phytochemical Analysis of *Mangifera indica* Leaf Extract

When evaluating the potential medical advantages of plants and determining the active principles that serve as stabilizing and capping agents during the manufacture of nanoparticles and nanocomposites, a phytochemical study is essential (Shaikh *et al.*, 2020). The major phytochemicals present in the *Mangifera indica* leaf extract were identified by the following standard protocol with slight modification (Gebreslassie & Eyasu, 2019).

Test for Alkaloids (Wagner's Test): In a test tube, three milliliters of *Mangifera indica* leaf extract were combined with five drops of Wagner's reagent. Alkaloids can be identified by the reddish-brown precipitate that forms after being shaken vigorously.

Test for Saponins (Froth Test): Distilled water (4 mL) and *Mangifera indica* leaf extracts (2 mL) were combined and swirled. The presence of saponins is confirmed by the creation of the stable foam.

Test for Flavonoids: The lead acetate reagent test was used to measure flavonoids. Consequently, 1 mL of the *Mangifera indica* leaf extract solution was mixed with a few drops of lead acetate solution. The flavonoids are identified by the production of a distinct yellow tint.

Test for Phenols and Tannins: Three milliliters of *Mangifera indica* leaf extract were combined with three drops of a 5% FeCl_3 solution. The presence of phenols and tannins is indicated by the development of a blue-black color after vigorous stirring.

3.5. Some Optimization Parameters for monodoped and codoped nanocomposites using *Mangifera indica* Leaf Extract.

Optimization is required to obtain nanoparticles and nanocomposites with good quality, stability, and appropriate size. As per the standards of previous reports, various parameters that regulate the experimental condition as being suitable for nanoparticle and nanocomposite synthesis were optimized by varying one parameter and keeping the other constant at a time to obtain the optimum values. Accordingly, to synthesized *Mangifera indica* Leaf Extract Mediated ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs and Ag-Ni/ZnO NCs, the effects of concentration, volume of *Mangifera indica* Leaf extract, reaction temperature and pH were optimised before synthesising nanoparticles and nanocomposites (Arun *et al.*, 2020).

3.5.1. Concentration

In this study, concentration optimization for ZnO NPs and the percentage ratio for Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs were done. Accordingly, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.05M, 0.1M, 0.2M, and 0.3M), AgNO_3 (1, 2, 3.4, 5%), $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (1, 3, 5, 6%) and AgNO_3 - $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ / $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (1, 2, 3, 4, 5, 6%) by keeping other parameters constant and 0.1M, 4%, 5% and 2%, 4%, 6% for ZnO NCs, Ag-ZnO NCs, Ni-ZnO NCs and Ag-Ni/ZnO NCs were optimum values respectively.

3.5.2. Volume of plant extract

The types of biomolecules found in plant extracts and the amount used have a significant impact on the synthesis of NPs and NCs from plant extracts. According to the earlier study, employing additional extracts can expedite the nanoparticle manufacturing process (Christopher, J. *et al.*, 2015). This is because the fluid has more chemical components that bind to the precursor to quickly and stabilize the synthesised nanoparticles. In order to study the effect of adding plant leaf extract on the synthesis, filtered extract was added with precursor solutions in between 10-70 mL *Mangifera indica* leaf extract ratios and 25mL for ZnO, Ag-ZnO NCs, Ni-ZnONCs and 30mL for Ag-Ni/ZnO NCs, were the optimum volume.

3.5.3. Temperature

Temperature is another important factor that affects the synthesis of metal oxide nanoparticles. To study the effect of temperature on the formation of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs and Ag-Ni/ZnO NCs, temperature suggested for the biosynthesis of metal oxide nanoparticles and nanocomposites were used between 30°C and 100°C at four different temperatures to obtain optimum temperature synthesis and keep the amount of percentage ratio of dopant precursor, volume of extract and pH constant (Christopher, J *et al.*, 2015). Reaction temperature of 90°C for ZnO NPs, 70°C for both Ag/ZnO NCs and Ni-ZnO NCs and 80°C for Ag-Ni/ZnO NCs are optimum values.

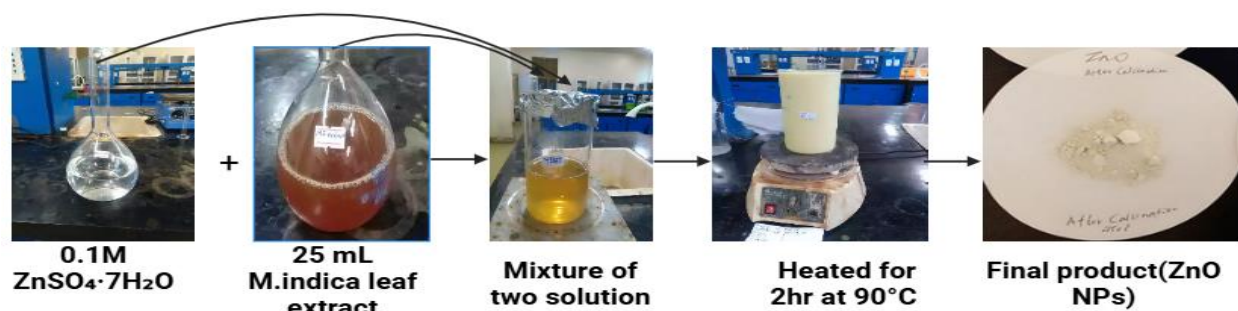
3.5.4. pH

The pH values of several plant extractions varied, requiring additional adjustments before the NPs and NCs could be biosynthesized. Additionally, it has been discovered that the activity of functional groups in plant extracts is influenced by pH. In this study, the pH of the solution was varied from 4 to 13 for ZnO NPs and Ag-ZnO NCs, whereas it was varied from 5 to 13 for Ag-Ni/ZnO NCs, i.e., pH 5.0, 7.0, 9.0, 11.0, and 13.0 were treated, and the reaction mixtures were adjusted by using HCl and NaOH (2M) (Gebremadin *et al.*, 2021). For ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs pH 11, 12, 11, and 11, respectively are optimum values.

3.6. Synthesis of nanoparticles and nanocomposites using *Mangifera indica* leaves extract.

3.6.1. Green Synthesis of ZnO NPs

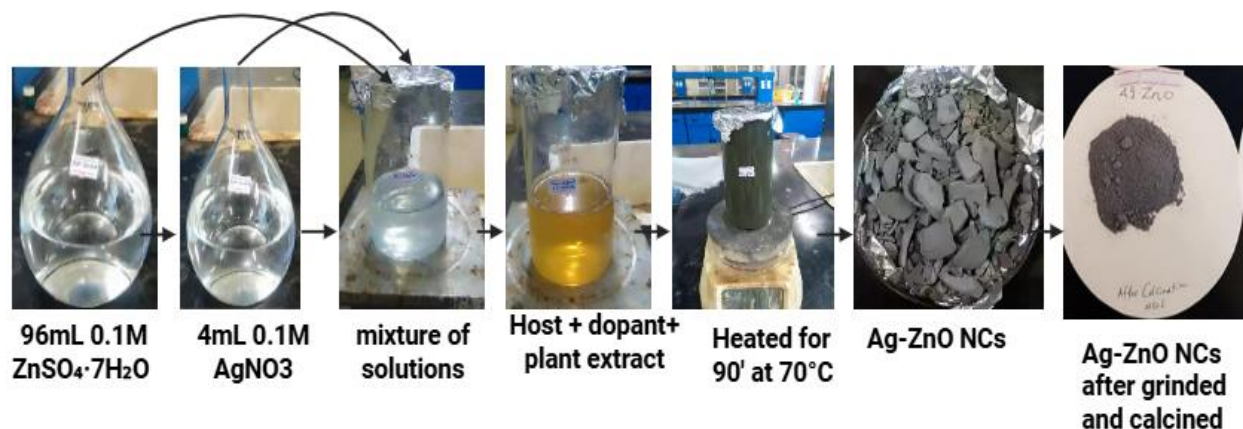
Zinc oxide nanoparticles were successfully synthesized using *Mangifera indica* leaf extracts. 100 mL of 0.1 M of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was added to 250 mL beaker containing 25 mL of *Mangifera indica* leaf extracts and stirred for 10 minutes at room temperature to homogenous. 2 M NaOH was added to the solution while stirring to bring its pH down to 11. Following two hours of constant stirring at 90°C , the creamy-colored precipitate solution was visible. After collecting the precipitate, it was centrifuged for five minutes at 10,000 rpm. The resulting precipitate was then cleaned multiple times using deionized water and absolute ethanol, collected, dried in an oven at 90°C for two hours, and ground with a mortar and pestle to a powder before being stored for additional physical analysis and biological uses.



Scheme 3. The synthesis method flow chart of ZnO NCs.

3.6.2. Green Synthesis of Ag-ZnO NCs

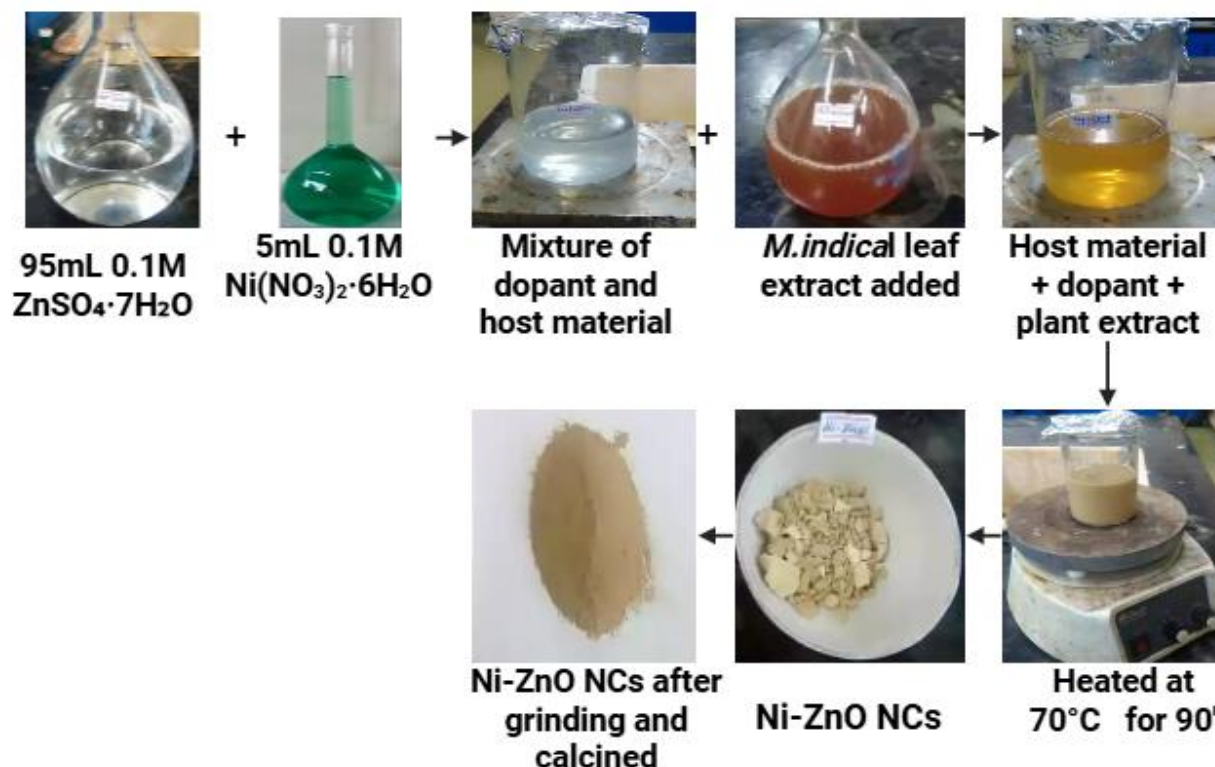
Ag-ZnO NCs were successfully synthesized according to (Khan *et al.*, 2022). Ag-ZnO NCs were successfully synthesized using *Mangifera indica* leaf extracts. In the synthesis of 4% Ag-ZnO, calculated volumes (4 mL) of AgNO_3 (0.1 M) were added dropwise to 96 mL of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1 M) solutions. In the meantime, 25 mL of a solution of *Mangifera indica* leaf extract was added dropwise, agitated for 10 minutes, and then NaOH (aq) (2 M) was added to raise the pH of the solution to 12. After that, the mixture was swirled for ninety minutes at 70°C . Centrifugation was then used to separate the brown precipitate for ten minutes at 10,000 rpm. After the filtrate was decanted to get rid of unwanted impurities, the precipitate was repeatedly cleaned with distilled water and then ethanol. The precipitate was then calcined for two hours at 450°C after being dried for two hours at 70°C . Lastly, the powder was kept for further use at 4°C .



Scheme 4. The synthesis method flow chart of Ag-ZnO NCs.

3.6.3. Green Synthesis of Ni-ZnO NCs

Ni-ZnO NCs was successfully synthesized using *Mangifera indica* leaf extracts according to (Khan *et al.*, 2022). In the synthesis of 5% Ni-ZnO NCs, calculated volumes (5 mL) of Ni $(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.1 M) were added dropwise to 95 mL of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1 M) solutions. In the meantime, 25 mL of a solution of *Mangifera indica* leaf extract was added dropwise, agitated for five minutes, and then NaOH (aq) (2 M) was added to raise the pH of the solution to 11. After that, the mixture was swirled for ninety minutes at 70°C. After that, centrifugation was used for 10 minutes at 10,000 rpm to separate the faded-yellow (green-yellow) precipitate. After the filtrate was decanted to get rid of unwanted impurities, the precipitate was repeatedly cleaned with distilled water and then ethanol. The precipitate was then calcined for two hours at 450°C after being dried for two hours at 70°C. Lastly, the powder was kept for further use at 4°C.

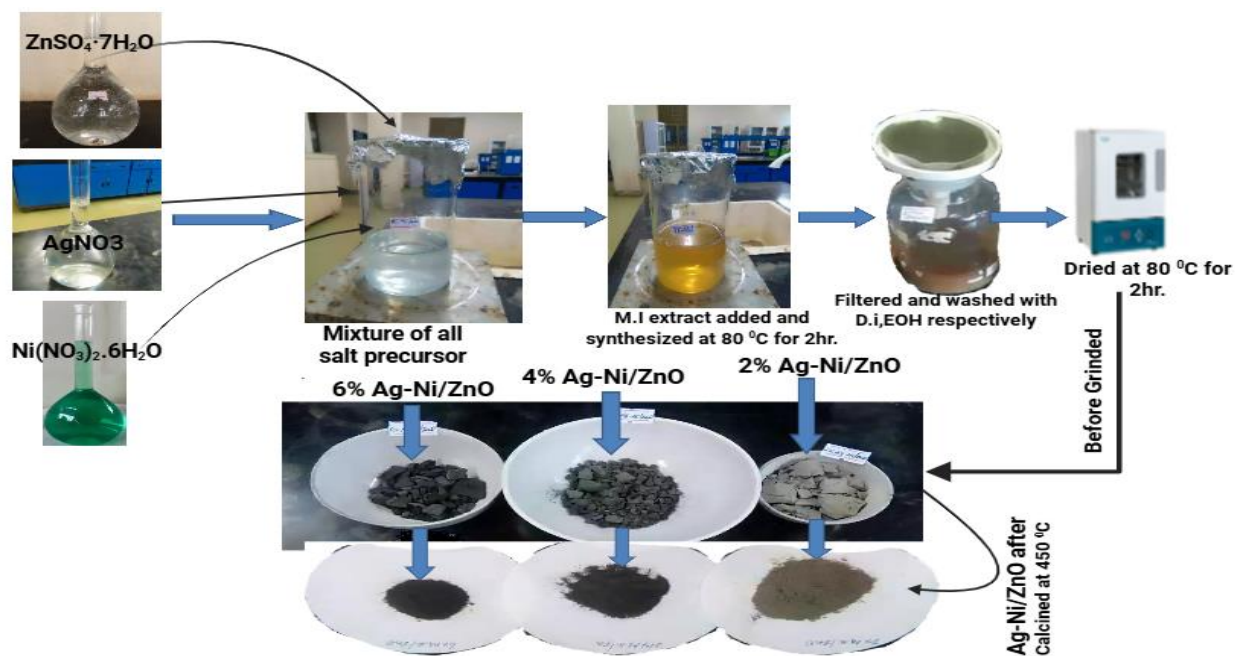


Scheme 5. The synthesis method flow chart of Ni-ZnO NCs.

3.6.4. Green Synthesis of Ag-Ni/ZnO NCs

Ag-Ni/ZnO NCs mediated by *Mangifera indica* leaf extract were successfully synthesized using (Ahmad *et al.*, 2019) protocol with some modification. In the synthesis of 2% Ag-Ni/ZnO NCs, 4% Ag-Ni/ZnO and 6 % Ag-Ni/ZnO as follows; for 2% Ag-Ni/ZnO NCs, calculated volumes of 2mL of 0.1M $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 2mL of (0.1 M) AgNO_3 were added dropwise to 250 mL beaker containing 96 ml of (0.1M) of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ solutions; for 4% Ag-Ni/ZnO NCs, 4mL of 0.1 M $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 4mL of (0.1 M) AgNO_3 were added dropwise to 250 mL beaker containing 92 ml of (0.1M) of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ solutions and for 6% Ag-Ni/ZnO NCs, 6mL of 0.1M $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 6mL of (0.1 M) AgNO_3 were added dropwise to 250 mL beaker containing 88 mL of (0.1M) of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ solutions. Meanwhile, 30 mL of *Mangifera indica* leaf extract solution was added dropwise to each of the percentages (2%, 4%, and 6%) separately, stirred for 10 minutes, followed by the addition of NaOH (aq) (2 M) to bring the solution pH to 11. Then, the solution was stirred for 120 minute at 80 0C. After that, the brown color for 2% Ag-Ni/ZnO NCs, the dark-brown color for 4% Ag-Ni/ZnO NCs, and the more intense dark-brown color (Scheme 6) for 6% Ag-Ni/ZnO NCs were formed.

As the percentage of the dopant increased, the color was changed from brown to dark brown with increased darkness for the maximum percent dopant. The precipitate was separated by centrifugation at 10,000 rpm for 10 min. To remove undesirable contaminants, the filtrate was decanted, and the precipitate was washed several times with distilled water and then ethanol. Then, the precipitate was dried at 80°C for 2h and then calcined at 450°C for 2h. Finally, the powder was stored at 4°C for further characterization.



Scheme 6. The synthesis method flow chart of Ag-Ni/ZnO NCs.

3.7. Characterizations of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs using *Mangifera indica* leaf extract.

3.7.1. UV-Vis Analysis

On a UV-Vis spectrophotometer, the electronic spectra of the ZnO NPs, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO NCs in solution were performed in the 200-800 nm range. The absorbance was recorded in a quartz cell at room temperature, and the band gap energy was calculated using the Tauc method.

The band gap energy (E_g) was calculated from the Tauc plot by using the Tauc/DavidMatt model (Eq. 2).

$$Ah\nu = \alpha (h\nu - E_g)^{n/2} \dots\dots\dots (2)$$

Where A, h, ν , α , and E_g are constant, Planck's constant, light frequency, absorption coefficient, and energy band gap, respectively.

Generally, surface plasmon resonance of nanoparticles and energy band gap of samples were analyzed by UV-Vis spectroscopy, and then graphs of absorbance versus concentration were plotted (Boddolla *et al.*, 2018).

3.7.2. FT-IR Analysis

Fourier transform infrared (FT-IR) spectra recorded in $4000\text{-}400\text{ cm}^{-1}$ were used to identify the presence of functional groups and to receive valuable information regarding the presence of ligand in the metal NPs (Abd El-Aziz *et al.*, 2019). The KBr pellet approach was used to identify the most likely biomolecule and nanoparticle produced using *M. indica* leaf extract. The FT-IR technique was used to obtain an infrared spectrum of absorption/emission of solids and gather spectral data in an extensive spectral range (Abd El-Aziz *et al.*, 2019).

3.7.3. XRD Analysis

XRD offers details on unit cell sizes, bond angles, chemical composition, and the crystallographic structure of natural and synthetic materials and is also utilized for the identification of crystalline materials. The crystallinity and crystalline phase of synthesized samples were determined through X-ray diffraction (XRD) in the 2θ range of 20 to 80 with a scan speed of $0.03^\circ/\text{min}$ using Cu-K α ($\lambda = 1.54\text{ \AA}$) radiation operated with an accelerating voltage and applied current of 40 kV and 30 mA, respectively, and the average crystallite size was calculated using the Debye-Scherrer equation (Eq. 3).

$$D = 0.94\lambda / \beta \cos\theta \dots\dots\dots (3)$$

Where D is the crystallite size, λ is the X-ray source wavelength, β is the full width at half-maximum (FWHM) of a peak, and θ is the Bragg diffraction angle (Thodeti *et al.*, 2018).

3.8. Antioxidant and Antibacterial test of nanoparticles and nanocomposites using *Mangifera indica* leaf extracts.

3.8.1. Antioxidant Activity Test

The antioxidant potential of the *Mangifera indica* leaf, ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCS and Ag-Ni/ZnO NCs (2%, 4%, 6%)) and the standard (ascorbic acid) was evaluated using the radical scavenging activity of stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to the method previously described with slight modifications (Khan *et al.*, 2023).

A 0.1 mM DPPH solution was made by dissolving 0.004 g of DPPH crystalline solid in 100 mL of analytical-grade methanol. Our biologically generated Ag-Ni/ZnO nanocomposite was tested for its antioxidant capacity using a standard ascorbic acid-DPPH assay method. A stock solution of 600 µg/mL of all samples (*Mangifera indica* leaf, ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCS and Ag-Ni/ZnO NCs (2%, 4%, and 6%)) and ascorbic acid were prepared in 10 mL methanol each. All samples and standards were prepared in methanol serially in five dilutions of various concentrations (500 µg/mL, 400 µg/mL, 300 µg/mL, 200 µg/mL, and 100 µg/mL) from the stock solution. From each of the diluted solutions (1 ml from each sample and standard), 2 ml from 0.1 mM methanolic DPPH solution (0.004 g of DPPH in 100 mL methanol) were mixed and shaken to homogeneity. After 30 minutes of being kept at room temperature in the dark, the absorbance of the samples, control, and standard was determined in relation to methanol at a constant wavelength of 517 nm using a UV-VIS spectrometer. Methanol was employed as a blank solution, methanol DPPH solution served as the control, and vitamin C served as a standard (positive control) (Khan *et al.*, 2023). Plotting the percentage scavenging versus concentration was used to determine the IC_{50} value. The IC_{50} values and % scavenging of the samples were compared with ascorbic acid, which is utilized as the standard, and the percentage scavenging activity at various doses was calculated. Each test was run three times, and the absorbance readings of the test and standard were compared to determine the % scavenging.

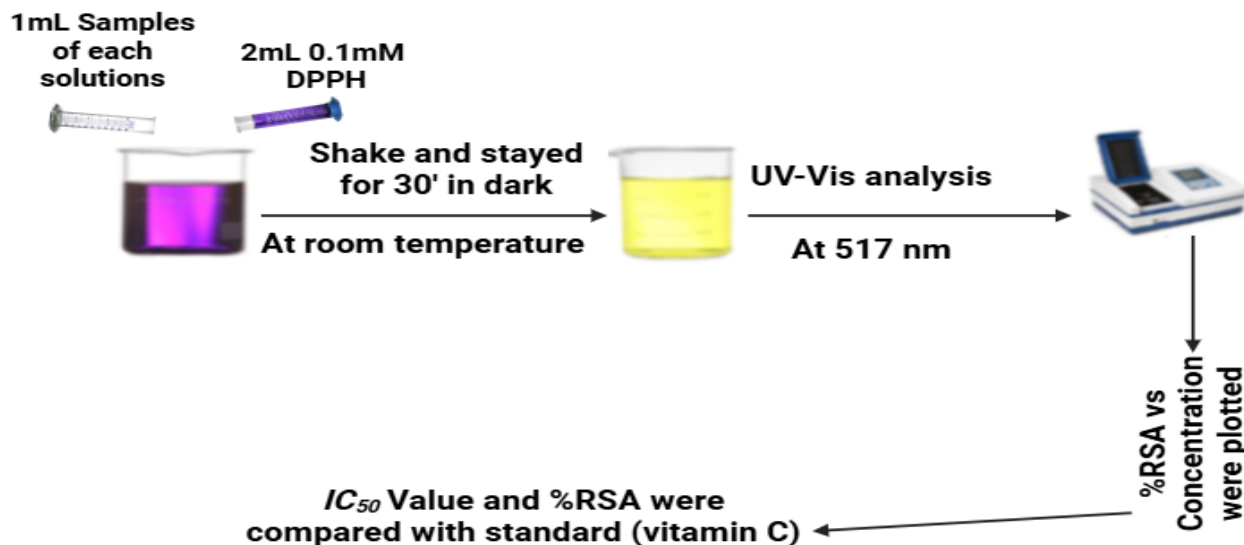
$$\text{DPPH \% Scavenging of samples} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \dots \dots \dots (4)$$

Where A_{Control} is the absorbance of 0.1 mM DPPH methanolic solution (without sample) and

A_{sample} is the absorbance of the mixture of sample and solution of 0.1 mM DPPH solution.

Pure solvent (methanol) was used as a blank (Hussen and Endalew, 2023). A linear regression equation was used to calculate the IC_{50} (Eq.5), with the sample concentration acting as the x-axis and the percent inhibition acting as the y-axis, using the regression equation $y = ax + b$, where a is slope of graph and b is the y-intercept (González Larraza *et al.*, 2020).

$$IC_{50} = \frac{50-b}{a} \dots\dots\dots (5)$$



Scheme 7. Schematic flow chart of antioxidant analysis.

3.8.2. Anti-bacterial Activity Test.

Two distinct bacterial varieties were used to test the antibacterial properties of nanomaterials made from *Mangifera indica* leaf extract: the microbiology laboratory at Wallaga University developed one gram-positive (*Staphylococcus auras*) and one gram-negative (*Escherichia coli*) bacterium based on their availability. About 300 mg of each of samples *Mangifera indica* leaf extract, ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCS, and Ag-Ni/ZnO NCs (2%, 4%, and 6%)) were dissolved in 1 mL of 10% DMSO solvent to get a stock solution of 300 mg/mL. Four serially diluted solutions (200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL) were made from the stock solution in order to observe the concentration-dependent effect, and the tests were conducted in accordance with established protocol (Takele *et al.*, 2023).

The antibacterial activity of the NPs and NCs was examined using the agar disk diffusion method. A bacterial suspension (1.0×10^8 CFU/ml) was then injected into standard Petri plates that had previously been prepared using Muller-Hinton agar in compliance with the manufacturer's instructions. A sterile cotton swab was used to swab little amounts of bacterial solutions onto each MHA plate. After that, the agar plate surface was streaked and seeded evenly. This procedure was carried out two or more times while streaking in order to produce a uniform dispersion of inoculums, and the agar's rim was swabbed at the conclusion (Takele *et al.*, 2023).

Sterilized filter paper discs with a 6 mm diameter were positioned evenly spaced throughout the seeded plate after being dipped into the NPs and NCs solutions. The infected plate was incubated for 24 hours at 37°C after being allowed to congeal for 30 minutes to allow for pre-diffusion time. On the plates, a zone of inhibition which appears as a distinct area surrounding the discs was sought after. To evaluate the antibacterial activity and compare it to the standard antibiotic, the diameter of the growth inhibition zone of each sample was measured using a ruler in millimeters (mm) after a 24-hour period. The disk was submerged in DMSO as a negative control, and 15 µg of ciprofloxacin was used as the positive control. The disk was submerged in DMSO as a negative control to ensure that neither the solvent nor the paper disk were responsible for any antibacterial activity (Takele *et al.*, 2023).

4. Statistical Data Analysis.

All data obtained from UV-vis, XRD, and FT-IR characterization of synthesized nanoparticles and nanocomposites was analyzed using Origin 8 software, and GraphPad Prism software to interpret the antioxidant activity of synthesized nanomaterials.

4. RESULTS AND DISCUSSIONS

4.1. Phytochemical analysis of *Mangifera indica* leaves extract

Using a different test, the findings of the phytochemical test, which was conducted to validate the different kinds of bioactive compounds present in the extract of *Mangifera indica* leaves, are shown in (Appendix 3). For the synthesis of nanomaterials with certain activities, it is desirable to know the correlation between a plant's phytoconstituents and bioactivity. An aqueous extract was created from the leaves of the *Mangifera indica* plant, yielding a brown-green extract. Therapeutic effectiveness is determined by the basis of the current study, which is to initiate research into the medicinal characteristics and potential curative qualities of the plant species *Mangifera indica* and its phytocompounds. According to the physical observation test, phenolic and flavonoids compounds were more prevalent than the other phytochemical compounds, followed by saponins, which were verified by the development of stable foam, and lower concentrations of alkaloids and tannins in the leaf extract of *Mangifera indica*. By conducting a variety of phytochemical assays, which verified the existence of various components to impart biological activities of the plant, the therapeutic potential of extracts was evaluated. Because of *Mangifera indica* leaf extracts include a wide range of phytochemicals, it can be inferred from this work that they have been crucial to the synthesis of NPs and NCs. According to earlier research, every phytochemical ingredient included in *Mangifera indica* leaf extract has been shown to have positive health effects on people (Maharaj *et al.*, 2022). (Table 3) provided a summary of the most significant bioactive components in *Mangifera indica* leaf extract. This outcome closely aligns with previous research (Kumar *et al.*, 2021).

Table 3. Phytochemical screening of the plant extracts.

S/No	Phytochemical	Chemical tests	Observation	Results
1	phenolic	ferric chloride	blue-black	+++
2	Alkaloid	Wagner`s test	Reddish-brown precipitates	+
3	saponins	frothing test	Stable Foam formation for	++
4	Tannin	ferric chloride	blue-black	+
5	flavonoid	alkaline reagent	Yellow precipitate	+++

+, ++ and +++ ...Indicates present, more and mostly present of phytochemicals respectively.

4.2. Optimization of reaction conditions.

4.2.1. Concentration of precursor

The volume of plant extract utilized must match the ratio of the precursor concentration. In this study, Concentration optimization for ZnO NPs, percentage ratio for Ag-ZnO NCs, Ni-ZnO NCs and Ag-Ni/ZnO NCs were done using (Mamo *et al.*, 2024) with minor modification. Accordingly, ZnSO₄·7H₂O (0.05M, 0.1M, 0.2M, and 0.3M), AgNO₃ (1, 2, 3.4, 5%), Ni (NO₃)₂·6H₂O (1, 3, 5, 6%) and AgNO₃- Ni (NO₃)₂·6H₂O/ ZnSO₄·7H₂O (1, 2, 3, 4, 5, 6%) at constant other parameter was done. The intensity of the absorption peak for ZnO NPs increased in tandem with the increase in metal salt solution concentration from 0.05 to 0.1 M. The intensity of the absorption peak decreased as the concentration was increased to 0.2 and 0.3 M. As a result, it was shown that the production of nanoparticles decreased when the concentration of metal ions was raised over the ideal threshold. This is most likely because larger concentrations of zinc precursor cause ZnO NPs to aggregate rather than form capped nanoparticles in a colloidal solution. Then, because it generated a sharp, strong, and redshift peak that indicated the creation of nanoparticles, 0.1 M was determined to be the ideal concentration for the manufactured ZnO NPs which is similar result with (Takele *et al.*, 2023).

Different percentage ratios of Ag-ZnONCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs (1%, 2%, 3%, 4%, 5%, and 6%) of Ag and Ni in 98%, 96%, 94%, 92%, 90%, and 88% of Zn were reacted with 30 mL leaf extracts, and Ag-ZnONCs (4%), Ni-ZnO NCs (5%), and Ag-Ni/ZnO NCs (2%, 4%, and 6%) were optimized. They created a distinct, intense peak that shows the creation of nanoparticles and nanocomposites because the intensity of the surface plasmon peak directly correlates with the concentrations of manufactured nanoparticles and nanocomposites in the solution. They also offered a UV-Vis absorbance value at the largest wavelength (red shift), which was associated with peak intensity and peak sharpness. Extracts from the leaves of *Mangifera indica* can therefore be employed as a stabilizing and reducing agent to produce a large number of widely dispersed NPs as the particle sizes rise. The outcome closely matched a recent publication, and a rise in intensity indicates the formation of additional nanoparticles (Firisa *et al.*, 2022).

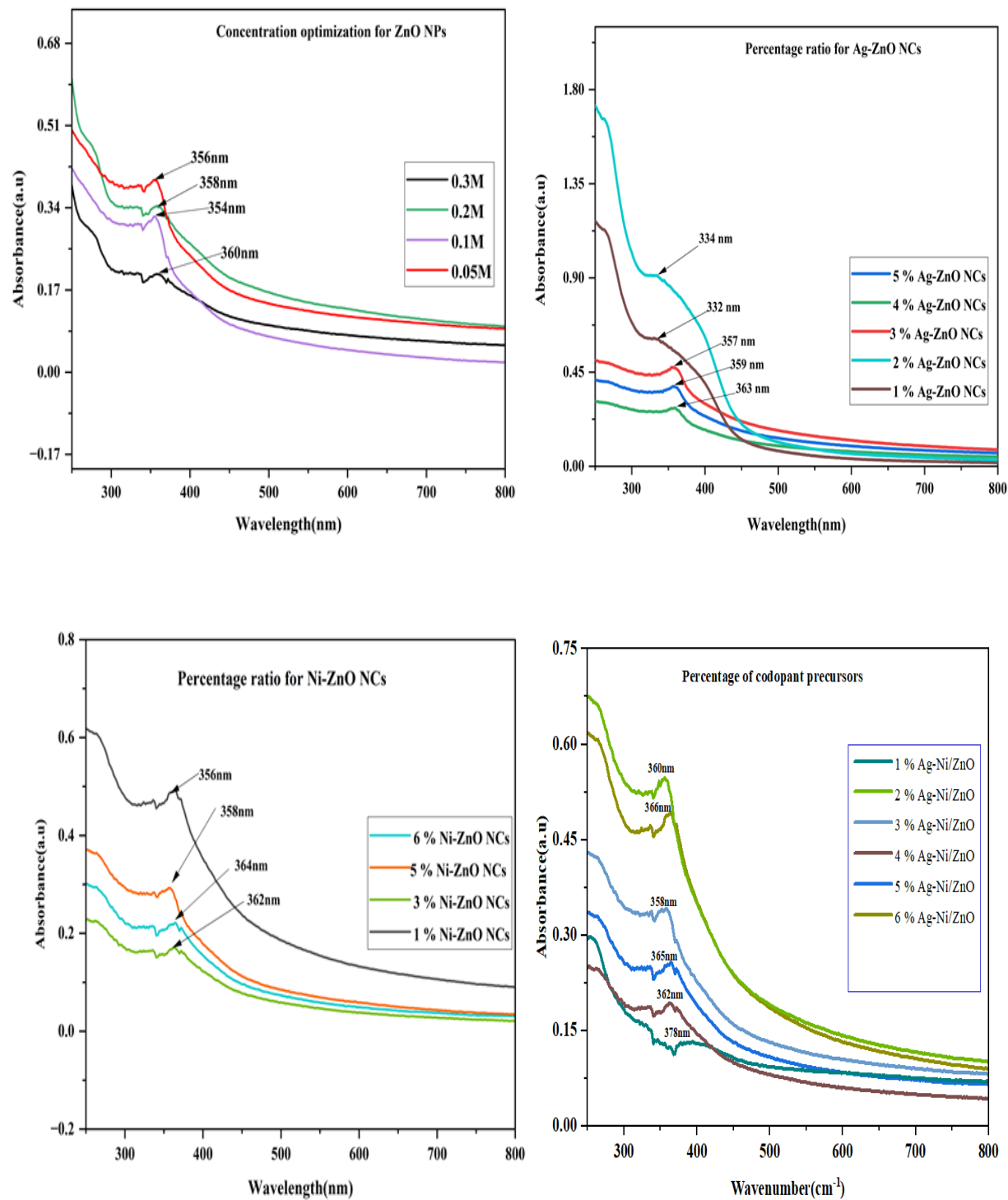


Figure 4. Concentration optimization of NPs and NCs.

4.2.2. Varying volumes of plant extract

The kinds of biomolecules included in plant extracts and the amount employed have the biggest effects on the synthesis of NPs utilizing plant extract. The reduction of metal ions to reduced metal in their oxidation number is significantly influenced by the volume of plant extracts utilized in the nanoparticle manufacturing process. The length of time needed to produce nanoparticles depends on the quantity of plant extracts employed. According to the earlier study, employing additional extracts can expedite the nanoparticle manufacturing process. This is because the solution contains more chemical components that bind to the precursor to quickly generate and stabilize nanoparticles. Different volumes (10, 30, 50, and 70 mL) were reacted at the set proportion of codopant precursors in order to determine the ideal volumes of plant extracts. In a similar manner, different volumes of *Mangifera indica* leaf extract (from 10 to 70 mL) were used, and the results showed that 25mL for ZnO, Ag-ZnO NCs, Ni-ZnO NCs and 30mL for Ag-Ni/ZnO NCs, were the optimum volume. The NPs and NCs mediated by *Mangifera indica* leaf extract provided the largest absorption peaks at this volume; hence, it was optimized by providing the smallest wavelength which match with (Rajendaran *et al.*, 2019). This was explained by the reaction between the phytochemicals in the plant extract and the precursor, which is what, causes the bio reduction and stabilization of the NPs.

When the amount of extract was increased from 10 mL to 25 mL and 30 mL, it was seen that the absorption and peak prominence improved. Then, because a strong peak with high intensity was achieved, 25 mL for ZnONPs, Ag-ZnO NCs, Ni-ZnONCs, and 30 mL for Ag-Ni/ZnO NCs were optimized. The absorption peak was reduced when the volume was greater or less than this amount. For reduction of metal ions in the reaction mixture and cap the surfaces of the NPs and NCs by preventing their agglomeration, bioactive compounds at a specific optimum value were sufficient.

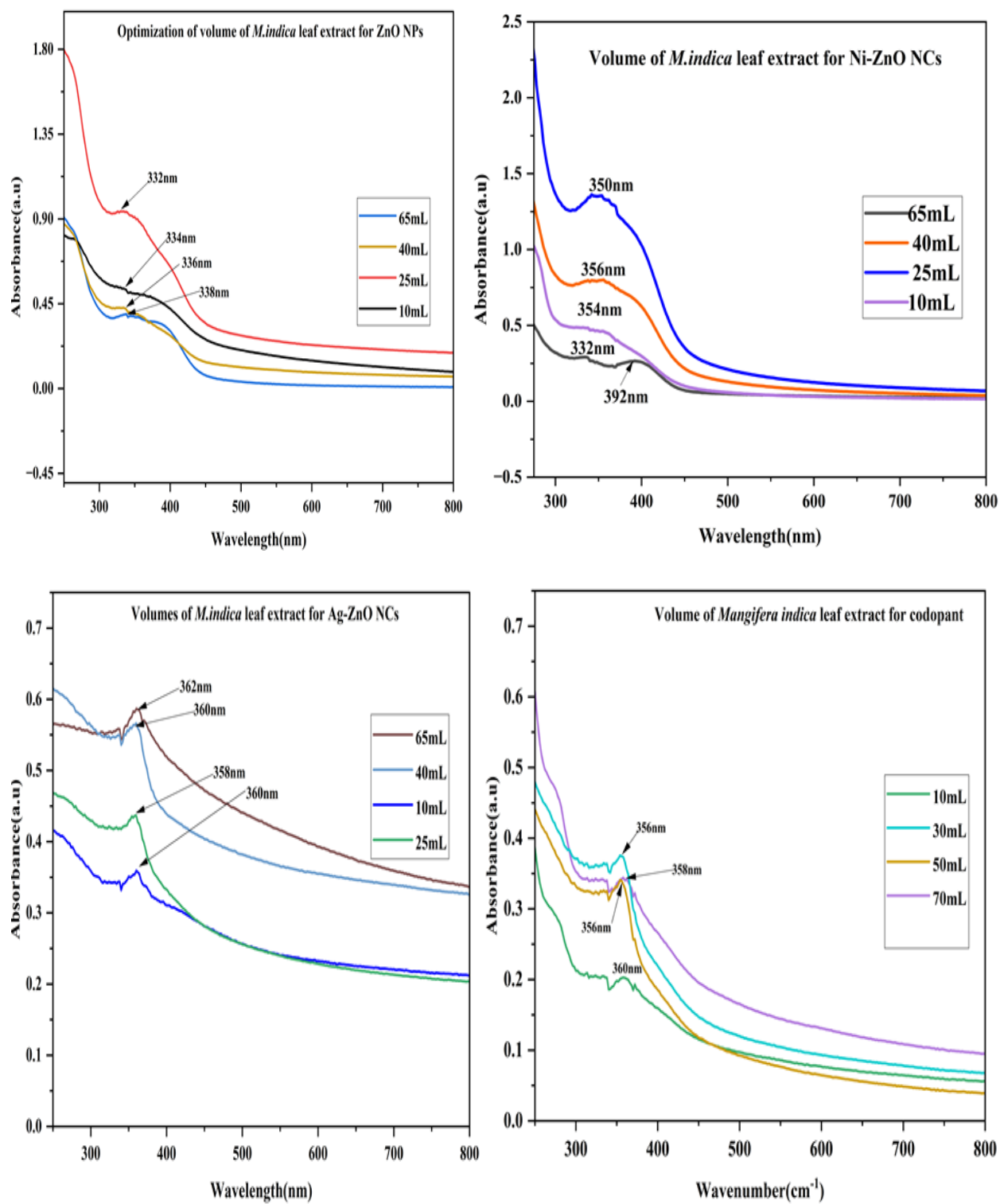


Figure 5. Optimization of volume *Mangifera indica* leaf extract for NPs and NCs

4.2.3. Effect of Temperature.

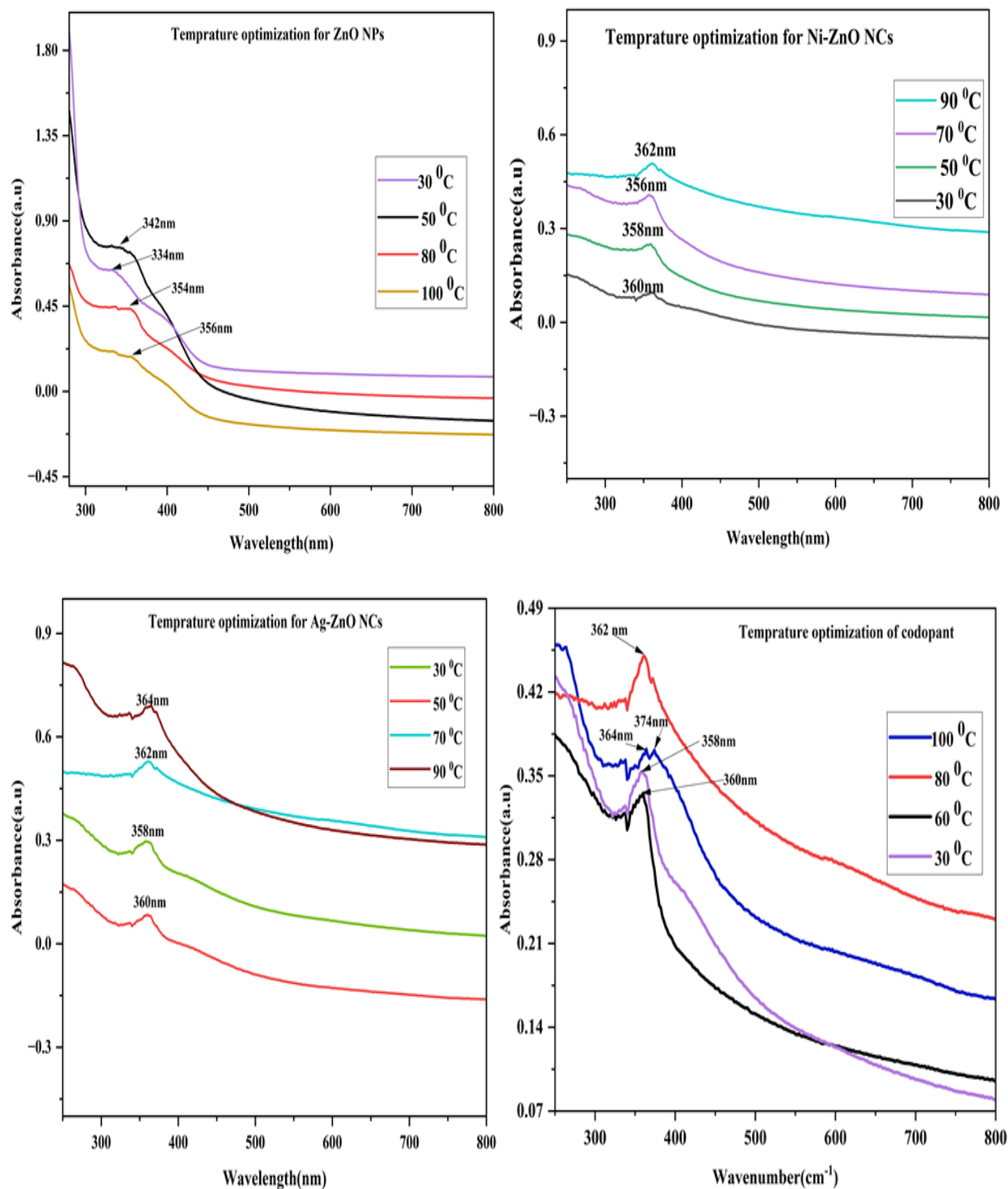


Figure 6. Temperature optimization of NPs and NCs

One significant element influencing the formation of metal oxide nanoparticles is temperature. For the biosynthesis of metal oxide nanoparticles and nanocomposites, the recommended temperature range was 30°C to 100°C at four distinct temperatures. This allowed for optimal temperature synthesis while maintaining a steady volume of extract, pH, and the percentage ratio of dopant precursor. Consequently, the optimum temperatures were 90°C for ZnO NPs, 70°C for both Ag/ZnO NCs and Ni-ZnO NCs and 80°C for Ag-Ni/ZnO NCs. The findings showed that when temperature rose, so did the intensity of the NP and NCs rise. This finding might be explained by the fact that metal ions rapidly reduce to their nanoparticles at higher temperatures. However, the intensity of the absorption peak decreased as the temperature was raised over the optimal level. The agglomeration of the NPs and NCs could also be the reason of this result, since the heat may have damaged the capping and reducing agents present in the plant extracts and similar idea were reported by (Rashid *et al.*, 2024).

4.2.4. Effect of pH

The pH of the solution was changed for ZnO NPs and Ag-ZnO NCs between 4 and 13 in order to get the ideal value, whereas it was adjusted for Ni-ZnO NCs and Ag-Ni/ZnO NCs between 5 and 13. For the acidic solution at pH=5, no surface plasmon resonance (SPR) band of nanoparticles developed. However, when the pH was adjusted to an alkaline state, the SPR band emerged at various wavelengths. The intensity of UV-Vis absorption increased as the pH of the reaction solution increased due to the increasing concentration of the produced nanoparticles and nanocomposites. High pH levels encourage the creation of nucleation sites and accelerate the reduction of zinc, silver, and nickel into their corresponding metal oxide NPs and NCs, which explains the synthesis of a sizable number of NCs. At pH 11, 12, 11, and 11, respectively, a high-intensity peak was detected by SPR for ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs. The remaining experiments were conducted at these pH levels after the obtained data led to the optimization of these values because of a better peak and maximum absorption peak intensity.

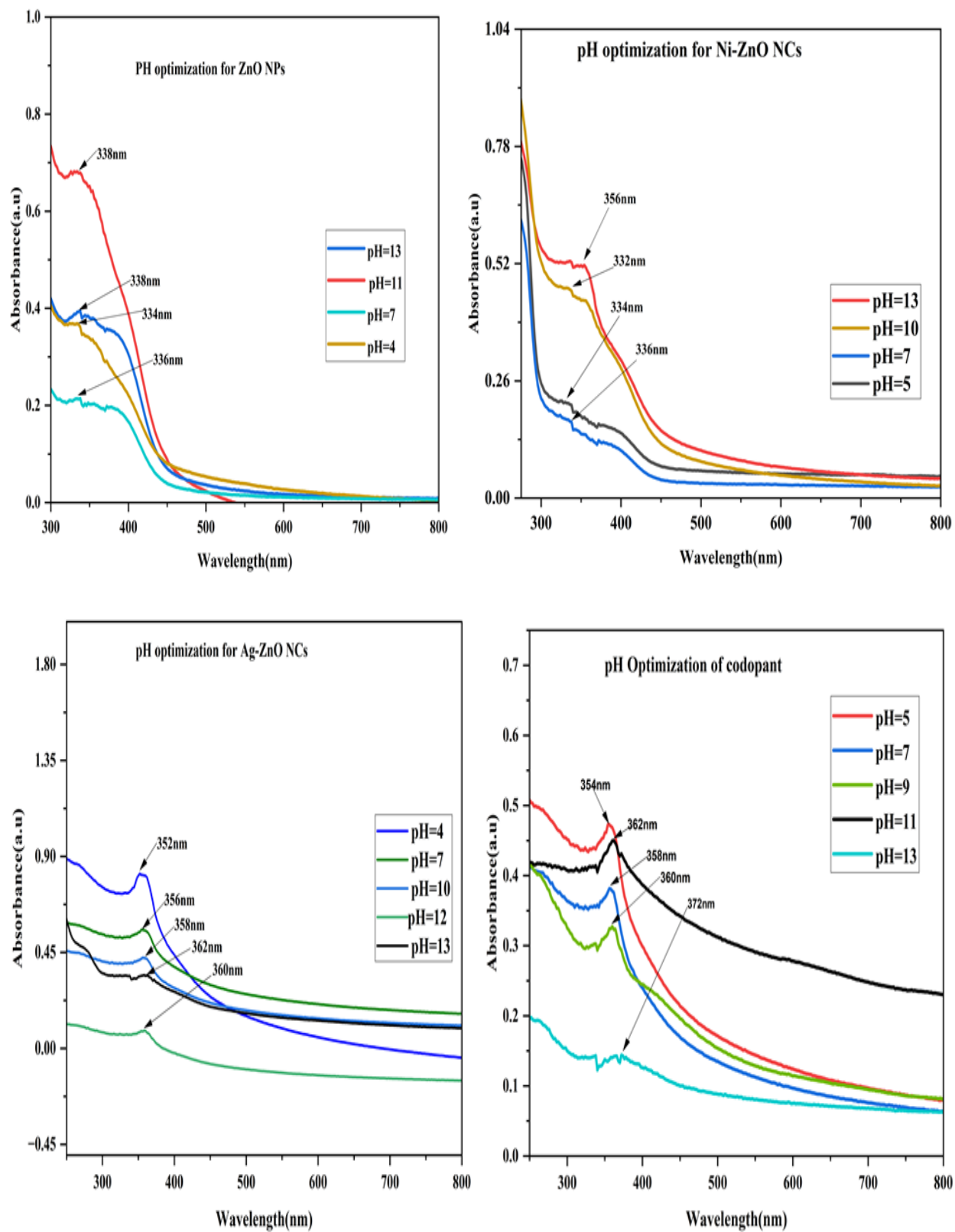


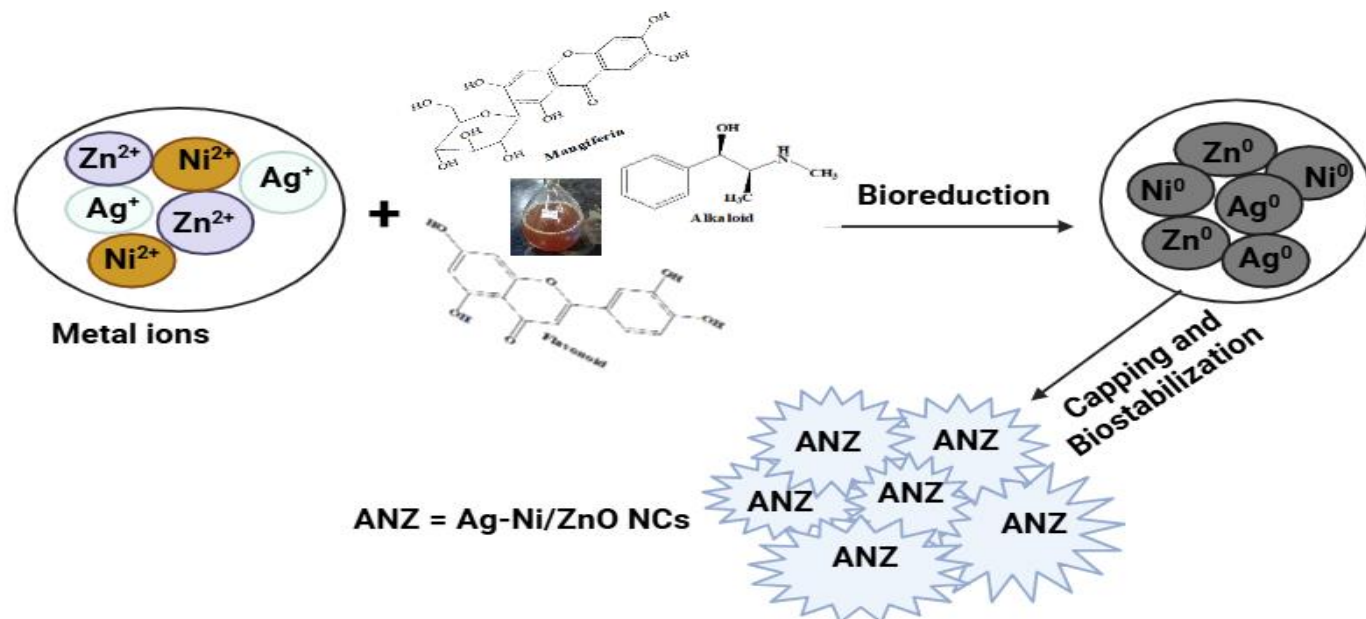
Figure 7. pH optimization of NPs and NCs

The pH was one of the factors influencing the size, shape, and composition nanoparticles. The effect of pH on the formation of Ag-Ni/ZnO NCs has been evaluated using UV-Vis spectroscopy. The pH of the aqueous medium can have a major effect on the pace of the metal ion reduction reaction. pH has an impact on the synthesis of NPs by altering the stabilizing and capping characteristics, which in turn affects the development of the nanoparticles. The presence of OH ions in an alkaline pH environment may enhance the reducing and stabilizing capabilities of the biomolecules in the leaf extract. The pH ranges of 5 to 13 were treated in order to determine the ideal pH value of NPs. The blue shift that was seen at pH 11 was caused by the particle size decreasing. Consequently, pH 11 was ideal. It was unable to create structurally changed Ag-Ni/ZnO NCs above and below pH 11, possibly because of the high OH ion concentration, which was supported by the poor absorption intensity. Because of the ionization of the phenolic group of extract, Ag-Ni/ZnO NCs develop more quickly at basic pH values than at acidic pH values which align with (Firisa *et al.*, 2022).

4.3. Synthesized NPs and NCs using *Mangifera indica* Leaf Extract.

Using an extract from *Mangifera indica* leaves, ZnO nanoparticles, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs were synthesized using the green technique. The extract's phytochemicals reduced the metal ions to their equivalent nanoparticles by acting as reducing agents. The addition of brown-green *Mangifera indica* leaf extract to a colorless zinc sulfate heptahydrate solution produced a bright green color that changed to yellow when 2M NaOH was added. After two hours of heating, a creamy-colored solid (precipitate) formed, signifying the formation of ZnO nanoparticles (Scheme 3). The mixture of colorless zinc sulfate heptahydrate and colorless silver nitrate turned a vivid green when the brown-green leaf extract of *Mangifera indica* was added. After the reaction time was completed, the color changed to a brown precipitate, indicating the formation of Ag-ZnO nanocomposites (Scheme 4). When the brown-green *Mangifera indica* leaf extract was added to the mixed solution of colorless zinc sulfate heptahydrate and green-colored nickel nitrate hexahydrate, it formed a bright green-colored solution, and after the reaction time was completed, the color changed to a faded yellow precipitate, indicating the formation of Ni-ZnO nanocomposites (Scheme 5). However, a mixed solution of colorless zinc sulfate heptahydrate, colorless silver nitrate, and green-colored nickel nitrate hexahydrate before the addition of *Mangifera indica* leaves extract forms a gray color.

When the brown-green *Mangifera indica* leaves extract was added, it form bright-green colored solution due to the domination of plant extract color over metal precursor solutions and after reaction completed, the color was changed to a brown, dark-brown, intense dark-brown precipitate for 2%Ag-Ni/ZnO NCs, 4%Ag-Ni/ZnO NCs and 6%Ag-Ni/ZnO NCs respectively indicating the formation of Ag-Ni/ZnO nanocomposites (Scheme 6).



Scheme 8. Mechanism for synthesis and stabilization of Ag-Ni/ZnO NCs using *Mangifera indica* leaf extract.

By taking into account the three stages, we can explain the mechanism of plant-mediated synthesis of metal and metal oxide nanoparticles (Scheme 8): the activation phase is when metal ions are reduced and reduced metal atoms undergo nucleation; the growth phase is when small adjacent NPs spontaneously coalesce into larger NPs, a process known as Ostwald ripening, which is when NPs are directly formed through heterogeneous nucleation and growth and further reduction of metal ions; and the termination phase determines the final shape of NPs. For metal oxide NPs, the final product is air-dried or calcined in air to obtain the final metal oxide NPs (Nagajyothi *et al.*, 2020).

4.4. Characterization of nanoparticle and nanocomposites.

4.4.1. Electronic spectra of the synthesized nanoparticles (UV-Vis spectroscopy)

One of the most popular, straightforward, and sensitive methods for identifying NPs and NCs formation is UV-Vis spectroscopy, which was used to characterize the *Mangifera indica* leaf extract, ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs. The measurements were made at room temperature between 250 and 800 nm.

The π - π^* transition of the conjugated C=C bonds in aromatic rings, which ranges between 230 and 320 nm, is the source of the peak in the UV-Vis spectra of *Mangifera indica* leaf extract at the high-energy short-wavelength region of 260 nm and 278 nm with close agreement with (Shi *et al.*, 2023). However, other authors (Bali *et al.*, 2018) reported that the UV-Vis spectra of *Mangifera indica* leaf extract occurred at a wavelength of 265 nm. Two absorption peaks were seen in the UV-visible absorption of *Mangifera indica* leaf extract (Figure 9). So, such absorptions may indicate the presence of different organic molecules in the leaf extract used for capping and reducing agent and formation of the nanocomposites. This value is consistent with the one that was previously reported (Gebishu *et al.*, 2022). The current analysis closely matches the other reported data for *Mangifera indica* leaf extract, which display a UV-visible absorption peak at 281 nm (Rotti *et al.*, 2022).

The initial sign of a successful ZnO NP synthesis was the yellow precipitate that resulted from adding plant extract to zinc sulfate heptahydrate in the presence of 2M NaOH. The presence of ZnO NPs is shown by an absorption peak at 354 nm in the UV-Vis spectrophotometric data (Figure 8). The production of ZnO NPs mediated by *Plectranthus amboinicus* leaf extract yielded similar results (Rajeshkumar *et al.*, 2018), while the produced ZnO NPs mediated by *P. granatum* peel extract likewise displayed a particular peak at 350 nm which is very similar result (Meshkatalasadat *et al.*, 2023). By extrapolating from the Tauc figure, the Eg of biosynthesized ZnO nanoparticles was determined to be 3.18 eV, which is consistent with (Sali *et al.*, 2021). Because of the modifications to the ZnO nanoparticles' band structure, a shift in the absorption peak is seen for the Ag-ZnO NCs. The absorption peak at 363 nm and (Figure 8) shows the matching band gap energy, which is 2.83 eV. Potential uses of Ag-ZnO NCs in optoelectronic devices are indicated by the observed shift of the absorption peak towards higher wavelengths and the reduction in the band gap brought on by silver doping.

After being doped with Ag^+ metal ions, E_g of biosynthesized ZnO NPs dropped from 3.18 eV to 2.83 eV. The dopant is primarily responsible for the decrease in the energy gap value, while the size and shape of the NCs may also have an impact. The energy gap decreases as a result of the acceptor impurity. By passing through the Fermi levels of the Ag clusters, the electrons from the valence band are stimulated to the conduction band (Sali *et al.*, 2021). The free excitons of ZnO nanostructures are responsible for the Ni-ZnO NCs, which are represented by an absorption band seen at about 358 nm. Absorption peaks seen in the wavelength range of 350-380 nm suggested the production of Ni-ZnO NCs, although other studies reported 358 nm for Ni-ZnO NCs (Mary *et al.*, 2022) (Figure 8). Due to the increased crystallinity of ZnO NPs, the band gaps E_{g1} and E_{g2} decrease from 3.18 to 3.1 eV (Figure 10), indicating that the Ag content has a greater effect on the energy bandgap structure of ZnO than the Ni content which aligns with previously reported literature (Ahmed *et al.*, 2019) and (Mohamed *et al.*, 2021). The bandgap energy data shows that Ni-ZnO NCs give 3.1 eV, which lowers the bandgap energy of ZnO NPs less than Ag-ZnO NCs.

For Ag-Ni/ZnO NCs (2%, 4%, and 6%), the corresponding UV-Vis spectroscopic measurements were 360, 362, and 366 nm. This is because extra energy levels on the edge of the conduction level are filled by Ag and Ni ions, which are incorporated into the lattice. The band gap of Ag-Ni-ZnO has decreased from 3.18 eV of pure ZnO NPs to 3.02, 2.92, and 2.72 eV for 2% Ag-Ni/ZnO, 4% Ag-Ni/ZnO, and 6% Ag-Ni/ZnO NCs, respectively, as a result of doping ZnO with Ni^{2+} and Ag^+ , which caused the mixing of 3d levels of these ions with 2p orbitals of O_2^- ions, creating a new energy level below the conduction band of ZnO. Due to the exchange interactions brought on by sp-d interactions between the band's electrons and localized d orbitals, the energy band and absorption edge of Ag-Ni/ZnO NCs displayed a red shift, demonstrating their capacity to collect visible light. This was only possible following the addition of noble metals to the ZnO semiconductor's lattice sites, which is consistent with published research (Sonia *et al.*, 2023) and (Naseer *et al.*, 2022).

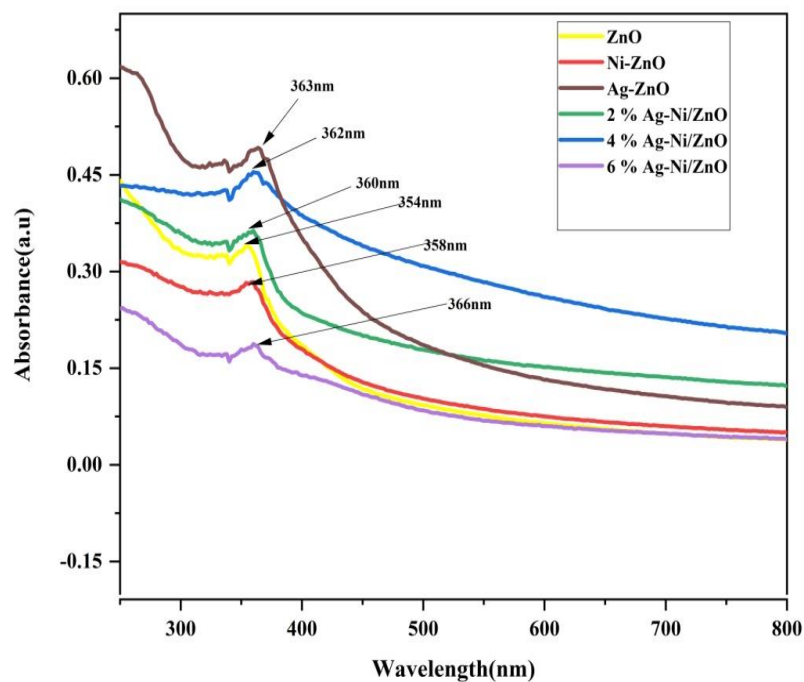


Figure 8. Uv-Vis spectra of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, 2% Ag-Ni/ZnO NCs, 4% Ag-Ni/ZnO NCs and 6% Ag-Ni/ZnO NCs.

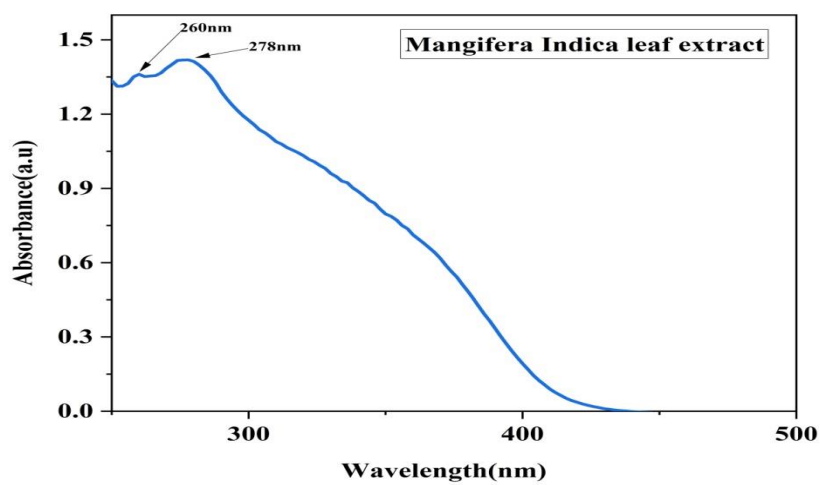


Figure 9. UV-Vis spectrum of *Mangifera Indica* leaf extract.

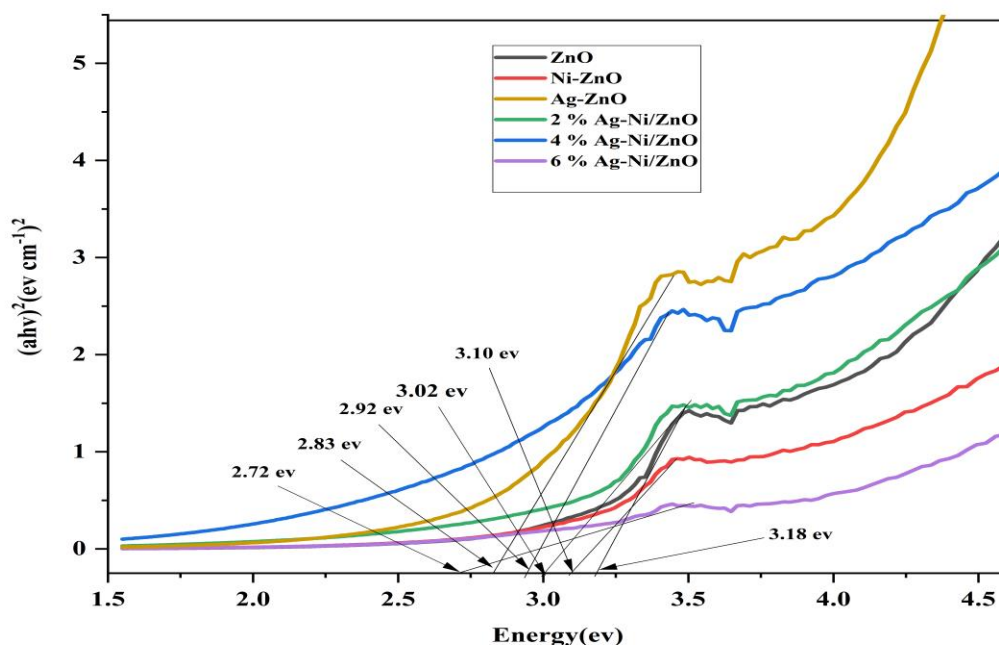


Figure 10. Optical bandgap of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs (2%, 4%, and 6%).

4.4.2. Fourier transforms infrared (FT-IR) spectroscopy

As shown in (Figure 11b), the FT-IR spectra of *Mangifera indica* leaf extract displayed numerous absorption peaks at 3228 cm^{-1} , 2929 cm^{-1} , 2171 cm^{-1} , 1602 cm^{-1} , 1446 cm^{-1} , 1371 cm^{-1} , 1238 cm^{-1} and 1033 cm^{-1} which reveals the presence of hydroxyl O-H stretching vibration of alcohol and phenol which is broad and strong peak, C-H stretching of alkane, weak $\text{C}\equiv\text{C}$ stretching of alkyne, $\text{C}=\text{C}$ alkene of aromatic ring, C-C stretching vibration of alcohol or carboxylic acid, O-C-O stretching of esters or may be due to the C-H stretching vibration of the alkene group, C-O ester group, -C-O(str.) of alcohol or phenol respectively which is consistent with (Rajeshkumar *et al.*, 2023). The presence of phytochemicals, including polyphenols, flavonoids, alkaloids, proteins, and amino acids, which are in charge of the production of NPs and NCs acting as reducing and capping agents, was verified by the FTIR spectrum. FTIR absorption peak of ZnO is similar to that of *Mangifera indica* extract except some peak shift and decrease in frequency (wavenumber) (Figure 14a). The peak around 2345 cm^{-1} is present in all samples except for plant extract, and it can be attributed to CO_2 absorbed by ZnO NPs either from the atmosphere or from the instruments.

The O-H stretching mode of hydroxyl groups, the amine-N-H stretching aromatic group, the O-C-O stretching of esters, or possibly the C-H stretching or the -C-O stretching peak for ether are represented by the bands shown at 3415, 1517, 1392, and 1120 cm^{-1} , respectively. The absorption peak found at 1120 cm^{-1} revealed the C-O group from the plant and 985 cm^{-1} and 862 cm^{-1} are C-H phase bending, whereas the peak at 688 describes the Zn-O cm^{-1} stretching mode of the zinc and oxygen bond, which arise from inter-atomic vibrations mainly between 1000-400 cm^{-1} (Alzahrani *et al.*, 2023).

The Ag-ZnO nanocomposites displayed peaks at different frequency and after deposition of Ag into ZnO NPs; the peak is shifted to lower frequency (Figure 14a). This significant shift is due to the formation of complexes related to Zn sites by the introduction of Ag into ZnO. The Ag-O and Zn-O vibrations are responsible for the vibration peaks at 873 and 680 cm^{-1} , respectively. The successful doping of the Ag ion in the ZnO lattice plane is confirmed by the presence of Ag^+ peaks at 873 cm^{-1} . The relative intensities of ZnO decline with small changes to the lower frequency following the deposition of Ag on the surface of ZnO NPs, which corresponds with (Zewudie *et al.*, 2023).

Table 4. Fourier transforms infrared spectra for ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, 2% Ag-Ni/ZnO NCs, 4% Ag-Ni/ZnO NCs, 6% Ag-Ni/ZnO NCs and *Mangifera indica* leaf extract.

Frequency(cm^{-1})	Assigned Bond	description
3300-3500	O-H	stretching mode of hydroxyl groups and phenolic group
2929	C-H	stretching vibration of alkanes
1390	C=C	-aromatic alkene
1602	-C=C, N-H	- indicates the aromatic C = C and free N-H stretching vibrations.
2171	-C-N - C $\equiv$ C	-the stretching of the C-N bond in the cyanide group or C $\equiv$ C in alkynes group
862	C-H	C-H rock, methyl, seen only in long chain alkanes
1238, 1033 and 1120	C-O	C-O stretching peak of ether group
2346	CO ₂	are attributed to carbondioxide molecules
1446	C-C	C-C stretching vibration of alcohol or carboxylic acid
1984	C=C=C	C=C=C stretching was observed at 1,984 cm^{-1}
1398	C-H	Stretching vibration of the alkene group.
1517	N-H	Stretching aromatic compounds from plant extract.
2167,2001	C $\equiv$ C	is the C $\equiv$ C stretching vibration
993,875,692	Ni-O, Ag-O, Zn-O	Due to (M-O) influenced by the bond strength and the masses of the atoms involved.
946-769	C-H	Carboxylic acid and aromatic bending.

Figure 14a shows the presence of distinctive peaks of the Ni-ZnO FTIR spectra. The introduction of Ni^{2+} causes the bond lengths of the Zn-O lattice to change when the Ni ion substitutes the Zn ion, shifting the vibration frequency of Zn-O to a higher frequency (873 cm^{-1} for ZnO-Ni and 684 cm^{-1} for ZnO-O). This demonstrates that Ni^{2+} ions have been added to the ZnO structure in place of Zn^{2+} ions. The Zn-O stretching mode is 684 cm^{-1} , while the absorption bands that appear at about 873 cm^{-1} are caused by vibrations of ZnO-Ni local bonds and defect states. This is related to (Sonia *et al.*, 2023).

The Zn-O stretching vibration peaks were shifted between 676, 679, and 692 cm^{-1} for 2% Ag-Ni/ZnO, 4% Ag-Ni/ZnO, and 6% Ag-Ni/ZnO, respectively. The vibration peaks at 869, 842, and 875 are for the Ag-O bond, and 991, 991, and 993 cm^{-1} are the Ni-O vibrational frequency shift of Ag-Ni/ZnONCs (2%, 4%, and 6%, respectively) which is in close agreement with (Pandiyar *et al.*, 2019). The peak for the ZnO shift higher frequency is due to the lattice distortion, which changes in the bond length by the substitution (or replacement) of Ni^{2+} or Ag^+ co-doped ions instead of Zn ions. There are no extra bands seen for Ag-Ni/ZnO NCs in the reported FTIR spectra, which suggest that there is no additional chemical bonding between Ag-Ni/ZnO NCs and pure ZnO NPs which is consistent with (Naseer *et al.*, 2022). The XRD data shown in (Figure 12) are consistent with the obtained result.

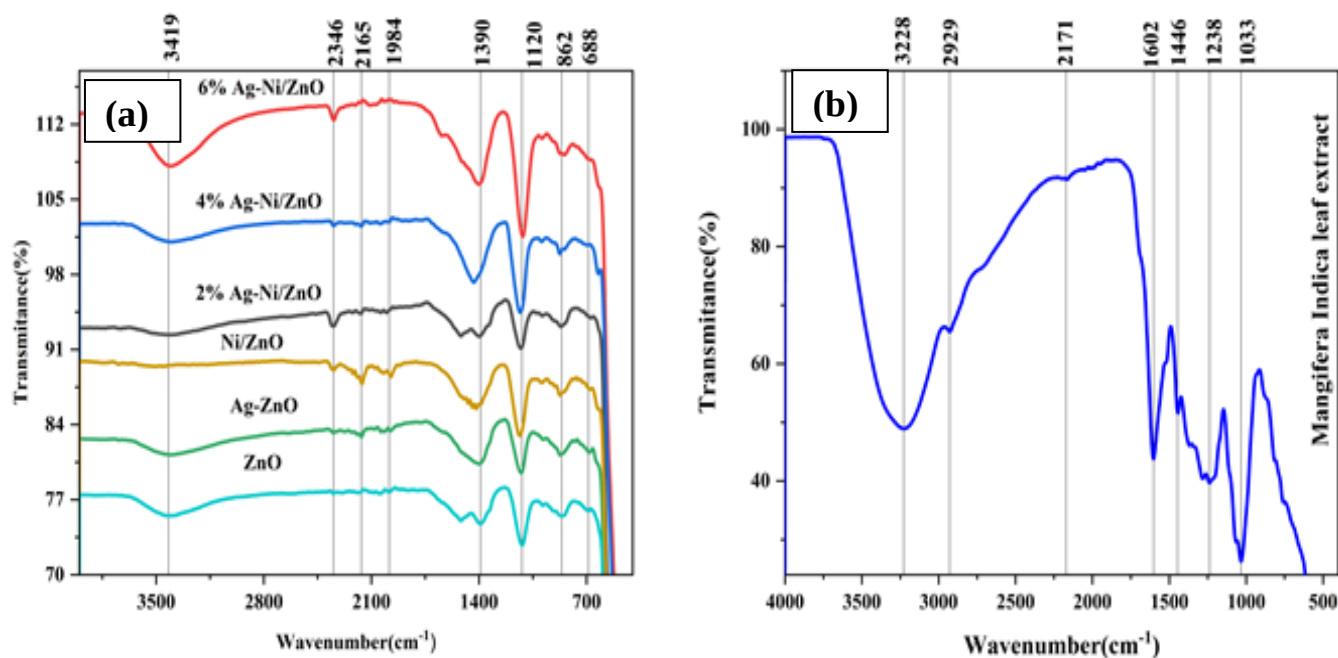


Figure 11. FTIR spectral absorption band of NPs and NCs (a) and *Mangifera indica* leaf extract (b).

4.4.3. X-ray diffraction (XRD) analysis of nanoparticles and nanocomposites.

ZnO NPs were subjected to XRD examination in order to ascertain their crystalline nature. The hexagonal wurtzite crystal structure of the ZnO nanoparticles was depicted in (Figure 12) by the XRD peaks obtained at 2θ values of 31.9° , 34.60° , 36.42° , 47.73° , 56.79° , 63.06° , 66.67° , 68.15° , 69.26° , 72.51° , 76.86° , and 81.31° , which corresponded to lattice planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202), and (104) according to the JCPDS card (No: 00-036-1451) and the JCPDS card (No: 01-080-0075).

Subsequent peaks at 31.92° , 34.22° , and 36.38° display Miller indices (100), (002), and (101), respectively, indicating the presence of a pure form of the hexagonal crystal structure of Zincite (ZnO) ZnO NPs. At 62.79° , 67.98° , and 68.90° , respectively, the following strong peaks in the XRD pattern with Miller indices (103), (112), and (201) may also be associated with zinc. Based on their full-width half maximum (FWHM) and peak location, the average size of the ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs was estimated using the Debye-Scherrer formula (Eq. 6).

$$(D = K\lambda/\beta\cos\theta) \dots\dots\dots (6)$$

where λ is the X-ray wavelength (0.15406 nm), D is the average particle size (in nm), K is the shape factor, and β is the full width at half maximum (FWHM). Using the aforementioned formula, the average crystal size of the nanoparticle was determined to be 18.51 nm, which is in close agreement with (Al-Ariki *et al.*, 2021).

Concerning with monodoped material, the Ag-ZnO NCs shows peaks that match both Ag and ZnO, demonstrating the synthesis of Ag-ZnO NCs (Figure 12). The crystal planes (100), (002), (101), (111), (200), (102), (110), (103), (220), (200), (112), (201), (004), (202), and (104), are represented by these patterns, which show peaks that appeared at 2θ values of 31.78° , 34.44° , 36.27° , 38.10° , 44.26° , 47.57° , 56.63° , 62.92° , 64.36° , 66.54° , 68.01° , 69.12° , 72.58° , 77.08° , and 81.56° . The face-centered cubic (fcc) structure of silver in Ag-ZnO NCs (JCPDF 00-001-1164) is represented by the three extra diffraction peaks (111), (200), and (220), which are situated at 38.10° , 44.26° , and 64.36° which align with reported literature (Makauki *et al.*, 2023).

The significant difference in ionic size between Ag^+ and Zn^{2+} ions in the ZnO system is the cause of this phenomenon and the production of ZnO appeared to be impacted by the presence of Ag metal. Since the ionic radii of Ag^+ (1.15 Å) are greater than those of Zn^{2+} (0.74 Å), the small shift of peaks to the lower angle and intensity decrement indicate the lattice expansion caused by partial substitution of Zn^{2+} ions by Ag^+ in the ZnO lattice. The wurtzite phase ZnO NP remains unchanged in the XRD examination following the deposition of Ag-ZnO NCs; nevertheless, the peak intensities have changed, confirming the creation of ZnO-Ag nanocomposites (Alzahrani *et al.*, 2023). When Ag ions were incorporated into ZnO, the average crystalline size of Ag-ZnO NCs was calculated to be 22.74 nm, which is in line with the published literature (Makauki *et al.*, 2023).

According to JCPDS card No. 36-1451, the XRD spectrum of Ni-ZnO NCs revealed peaks at 2θ values of 31.84, 34.50, 36.33, 47.64, 56.69, 62.97, 66.58, 68.06, 69.18, 72.67, 79.10, and 81.31° that corresponded to the lattice plane of (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202), and (104). According to previous publications (Azfar *et al.*, 2020), no new NiO diffraction pattern was seen, suggesting that Ni crystals were dispersed over the ZnO surface. Furthermore, the X-ray diffractograms show that Ni/ZnO NCs exhibit diffraction patterns that are exactly the same as those of ZnO NPs due to the closer ionic radius between Ni^{2+} (0.69 Å) and Zn^{2+} (0.74 Å). This could be the unmistakable proof that Ni was successfully incorporated into the ZnO NPs lattice. Furthermore, it has been noted that Ni/ZnO NCs samples exhibit a minor shift in diffraction peaks to a lower angle compared to pure ZnO NPs. Vegard's law states that although a dopant ion does not show a diffraction peak, it is in charge of shifting the host material's diffraction peaks. It is also observed that as the Ni concentration increases, the peak height of the diffraction peaks decreases noticeably, suggesting that the crystalline character of ZnO NPs is diminished with Ni impurity. With the addition of Ni ions to ZnO NPs, the computed average crystalline size was 19.91 nm, which is consistent with the published literature (Al-Araki *et al.*, 2021). With the exception of slight variations in diffraction angle and intensity, all of the diffraction peaks for Ni-ZnO NCs, Ag-ZnO NCs, and Ag-Ni/ZnO NCs (2%, 4%, and 6%) resemble that of ZnO NPs.

Three new diffraction peaks (111), (200), and (220) at 38.10° , 44.26° , and 64.36° appeared for Ag-ZnO NCs, 38.08° , 44.28° , and 64.56° for 2% Ag-Ni/ZnO NCs, 38.16° , 44.33° , and 64.51° for 4% Ag-Ni/ZnO NCs, and 38.18° , 44.28° , and 64.42° for 6% Ag-Ni/ZnO NCs (JCPDF 00-001-1239). We added equal molar amounts of Ni and Ag (2%, 4%, and 6%) to ZnO NCs in order to investigate the co-effect of Ni and Ag in ZnO NPs. In comparison to pure ZnO NPs, the diffraction peak intensity and diffraction angle decreased.

The crystalline quality of the samples is shown by the (1 0 1) peak intensity, which increases linearly as the amount of Ag-Ni codopants in ZnO increases. The addition of Ag-Ni codopant in ZnO, which results from the incorporation of Ag^+ and Ni^{2+} ions into the ZnO lattice, likewise shifts the high-intensity peak toward the higher angle side, as seen in (Figure 12). A little shift towards lower angles in the 2θ position was noted for Ag-ZnO and Ni-ZnO NCs. This implies that the difference in ionic radius between the dopant and host metal oxide (ZnO) structures is the cause of the 2θ shift. But in the case of Ni-ZnO NCs, Ni was unable to create a new phase on XRD because the ionic radii of Zn^{2+} ($0.74 \text{ \AA}$) and Ni^{2+} ($0.69 \text{ \AA}$) are nearly identical. In contrast to pure ZnO NPs, the insertion of Ag-Ni into ZnO caused a drop in the intensity peak of the diffraction peaks of Ag-Ni/ZnO NCs, indicating a loss in crystallinity. The average crystallite size of Ag-Ni/ZnO NCs (2%, 4%, and 6%) increased from 24.08 nm, 24.55 nm, and 25.13 nm, respectively, from 18.51 nm of ZnO NCs. The substitution of oxygen at Ag-Ni ions in the ZnO sites is the cause of the variation in average crystallite size which match with reported literature (Al-Arifi *et al.*, 2021).

Table 5. FWHM, diffraction angle, and average crystalline size of *Mangifera indica* leaf extract-mediated ZnO NPs, Ni-ZnO NCs, and Ag-ZnO NCs.

Peak No	ZnO				Ni-ZnO				Ag-ZnO			
	β	2 θ	hkl	D ave	β	2 θ	hkl	D aver	β	2 θ	hkl	D ave
1	0.59	31.94	(100)	18.51	0.56	31.84	(100)	19.91	0.57	31.78	(100)	22.74
2	0.53	34.60	(002)		0.62	34.50	(002)		0.56	34.44	(002)	
3	0.60	36.42	(101)		0.69	36.33	(101)		0.60	36.27	(101)	
4	0.73	47.73	(102)		0.51	47.64	(102)		0.50	36.27	(111)	
5	0.62	56.79	(110)		0.69	56.69	(110)		0.56	44.26	(200)	
6	0.80	63.06	(103)		0.68	62.97	(103)		0.44	47.57	(102)	
7	0.69	66.67	(200)		0.86	66.58	(200)		0.49	56.63	(110)	
8	0.70	68.15	(112)		0.98	68.06	(112)		0.52	62.92	(103)	
9	0.87	69.26	(201)		0.63	69.18	(201)		0.52	66.54	(220)	

Table 6. FWHM, diffraction angle, and average crystalline size of Ag-Ni/ZnO NCs (2%, 4%, and 6%).

Peak No	2%Ag-Ni/ZnO				4%Ag-Ni/ZnO				6%Ag-Ni/ZnO			
	β	2 θ	hkl	D average	β	2 θ	hkl	D average	β	2 θ	hkl	D average
1	0.41	31.75	(100)	24.08	0.38	31.73	(100)	24.55	0.38	31.82	(100)	25.13
2	0.22	34.42	(002)		0.36	34.38	(002)		0.31	34.47	(002)	
3	0.40	36.25	(101)		0.36	36.21	(101)		0.40	36.30	(101)	
4	0.82	38.08	(111)		0.50	38.16	(111)		0.55	38.18	(111)	
5	0.58	44.28	(200)		0.43	44.33	(200)		0.60	44.38	(200)	
6	0.56	47.56	(102)		0.58	47.53	(102)		0.55	47.61	(102)	
7	0.66	56.61	(110)		0.50	56.59	(110)		0.48	56.68	(110)	
8	0.705	62.90	(103)		0.62	62.87	(103)		0.57	62.95	(103)	
9	0.66	64.56	(220)		0.52	64.51	(220)		0.60	64.42	(220)	
10	0.74	66.50	(200)		0.59	66.47	(200)		0.82	66.56	(200)	
11	0.45	67.99	(112)		0.66	67.96	(112)		0.56	67.04	(112)	
12	0.76	69.11	(201)		0.70	69.09	(201)		0.61	69.16	(201)	
13	0.50	72.64	(004)		0.69	72.13	(004)		0.33	72.16	(004)	

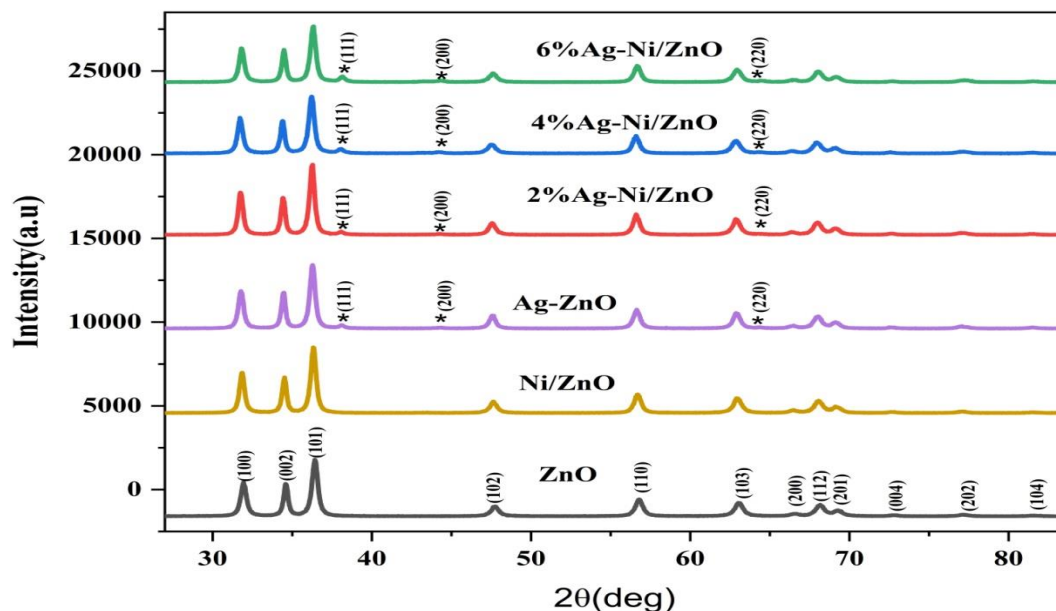


Figure 12. XRD pattern of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs (2%, 4% and 6%).

4.5. Antioxidant activity of nanoparticles and nanocomposites using *Mangifera indica* leaf extract.

Free radicals are bad for human health because they destroy and alter cells and antioxidants used to remove ROS. The in vitro antioxidant activity of nanocomposites has demonstrated their pharmacological relevance. The DPPH assay is a quick, easy, sensitive, and extremely promising technique for lowering or eliminating free radicals in order to examine the antioxidant capacity of nanoparticles (Noohpisheh *et al.*, 2020).

The IC_{50} values and %RSA of each NPs and NCs were determined and noted in (Table 7 and 8). The percentage radical-scavenging activity of synthesized nanocomposite and nanoparticle was examined at various doses of 100, 200, 300, 400, and 500 $\mu\text{g/mL}$. The phytochemical component of polyphenols has considerable antioxidant activity because it absorbs and neutralizes free radicals, quenches singlet and triplet oxygen, and caps the surfaces of nanoparticles and nanocomposites, which naturally have antioxidant activity. The standard's % radical scavenging activity at the maximum concentration (ascorbic acid) and the leaf extract of *Mangifera indica* was 97.84 ± 0.00 and 62.38 ± 0.00 , respectively.

According to the findings, *Mangifera indica* leaf extract has scavenged free radicals present in DPPH because it contains significant bioactive compounds with a variety of biological characteristics, such as antioxidant capabilities, which are useful in the treatment of a number of illnesses (Khan *et al.*, 2018) (Table 7 and 8). According to a phytochemical examination, the leaf extract of *Mangifera indica* is a rich source of steroids, ascorbic acid, saponins, and phenolic compounds. These substances possess exceptional antioxidant qualities.

Table 7. The DPPH radical scavenging activity of the *Mangifera indica* leaf extract, ZnO NPs, Ni-ZnO NCs, Ag-ZnO NCs and ascorbic acid.

Sample name	Conc. (µg/mL)	Average Absorbance	% DPPH Inhibition	IC ₅₀ (µg/mL)	Standard (Ascorbic acid)		
					Average Absorption	% DPPH Inhibition	IC ₅₀ (µg/mL)
<i>Mangifera indica</i> leaf extract	100	0.48±0.00	38.00±0.00	57.9	0.26±0.00	66.36±0.02	42.2
	200	0.44±0.00	44.02±0.03		0.20±0.00	73.46±0.04	
	300	0.40±0.00	49.20±0.00		0.14±0.00	81.68±0.01	
	400	0.35±0.00	55.19±0.00		0.07±0.00	90.21±0.04	
	500	0.29±0.00	62.38±0.00		0.01±0.00	97.84±0.00	
ZnO	100	0.48±0.00	39.03±0.25	51.25	0.26±0.00	66.36±0.02	42.2
	200	0.42±0.01	45.96±0.01		0.20±0.00	73.46±0.04	
	300	0.38±0.01	51.67±0.02		0.14±0.00	81.68±0.01	
	400	0.32±0.02	59.04±0.00		0.07±0.00	90.21±0.04	
	500	0.26±0.02	67.02±0.00		0.01±0.00	97.84±0.00	
Ni-ZnO	100	0.46±0.00	41.59±0.00	48.3	0.26±0.00	66.36±0.02	42.2
	200	0.40±0.00	49.32±0.004		0.20±0.00	73.46±0.04	
	300	0.33±0.00	58.19±0.003		0.14±0.00	81.68±0.01	
	400	0.253±0.00	67.75±0.003		0.07±0.00	90.21±0.04	
	500	0.17±0.000	77.57±0.00		0.01±0.00	97.84±0.00	
Ag-ZnO	100	0.43±0.00	44.45±0.00	46.5	0.26±0.00	66.36±0.02	42.2
	200	0.37±0.00	52.90±0.00		0.20±0.00	73.46±0.04	
	300	0.29±0.00	62.11±0.00		0.14±0.00	81.68±0.01	
	400	0.21±0.00	72.31±0.00		0.07±0.00	90.21±0.04	
	500	0.14±0.00	81.75±0.00		0.01±0.00	97.84±0.00	

Accordingly, the chemicals in *Mangifera indica* leaf extract increase antioxidant activity of the produced nanoparticles and nanocomposite however; it is not as active as ZnO NPs. According to other sources, *Allium sativum* leaf extract, which was utilized to create ZnO NPs, has 60% RSA antioxidant activity (Benshiga *et al.*, 2024) which is less activity than current study.

Table 8. The DPPH radical scavenging activity of Ag-Ni/ZnO NCs (2%, 4%, 6%) and ascorbic acid.

Sample name	Conc. (µg/mL)	Average Absorbance	% DPPH Inhibition	IC ₅₀ (µg/mL)	Standard (Ascorbic acid)		
					Average Absorption	% DPPH Inhibition	IC ₅₀ (µg/mL)
2% Ag-Ni/ZnO	100	0.37±0.00	52.54±0.02	45.7	0.26±0.00	66.36±0.02	42.2
	200	0.31±0.00	59.94±0.04		0.20±0.00	73.46±0.04	
	300	0.25±0.00	67.93±0.00		0.14±0.00	81.68±0.01	
	400	0.19±0.00	75.53±0.00		0.07±0.00	90.21±0.04	
	500	0.12±0.00	83.98±0.00		0.01±0.00	97.84±0.00	
4% Ag-Ni/ZnO	100	0.36±0.00	54.30±0.04	43.6	0.26±0.00	66.36±0.02	42.2
	200	0.29±0.01	62.60±0.02		0.20±0.00	73.46±0.04	
	300	0.24±0.00	69.21±0.00		0.14±0.00	81.68±0.01	
	400	0.17±0.01	77.81±0.00		0.07±0.00	90.21±0.04	
	500	0.10±0.00	86.34±0.05		0.01±0.00	97.84±0.00	
6% Ag-Ni/ZnO	100	0.33±0.00	57.39±0.04	41.3	0.26±0.00	66.36±0.02	42.2
	200	0.26±0.00	66.00±0.00		0.20±0.00	73.46±0.04	
	300	0.19±0.00	75.15±0.00		0.14±0.00	81.68±0.01	
	400	0.12±0.00	84.46±0.00		0.07±0.00	90.21±0.04	
	500	0.06±0.00	92.36±0.00		0.01±0.00	97.84±0.00	

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging experiment was used to assess the antioxidant activity of the produced ZnO NPs, as indicated in (Table 7). When dissolved in organic solvents, the stable free radical DPPH takes on a dark violet hue. By taking an electron or hydrogen from the antioxidant species, the DPPH radical is decreased, turning light yellow and causing a significant decrease in the absorbance maximum (Yadesa *et al.*, 2024).

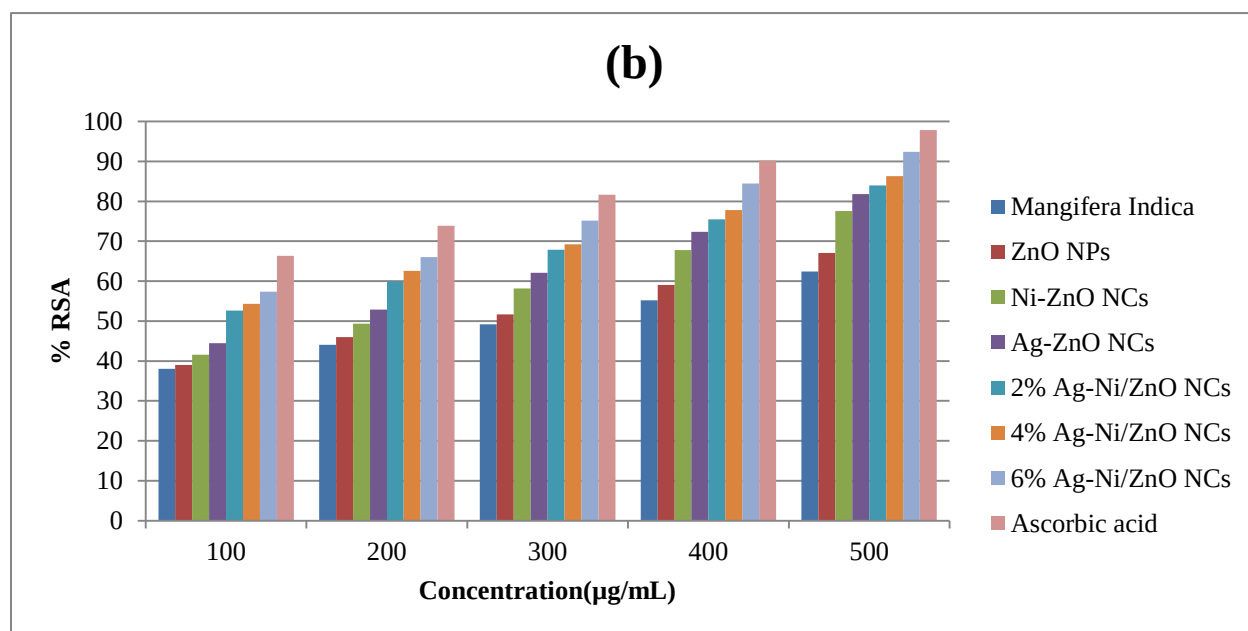
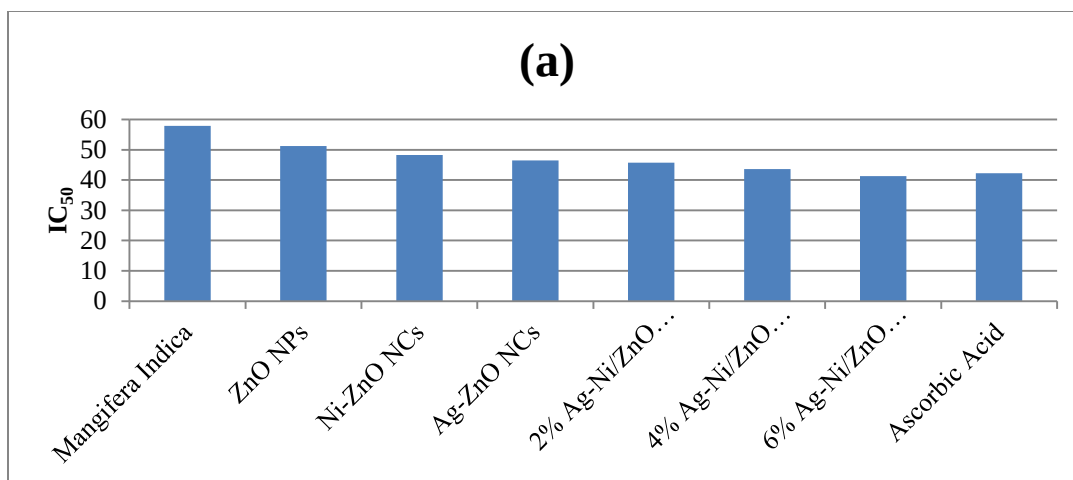


Figure 13. Bar graph representations of A) IC_{50} value and B) % RSA for *Mangifera indica*, ZnO NPs, Ni-ZnO NCs, Ag-ZnO NCs and Ag-Ni/ZnO NCs (2%, 4% and 6%) and ascorbic acid.

Here, a hydrogen atom from the supplied antioxidant is absorbed, reducing the unpaired electron of the nitrogen atom in the DPPH to the corresponding hydrazine. The potential of the synthesized ZnO NP samples as free radical scavengers was demonstrated by a decrease in DPPH absorbance at 517 nm (Yadesa *et al.*, 2024).

The water molecules are broken down into hydrogen and hydroxyl radicals by the high redox potential of ZnO, which stabilizes the DPPH free radicals and prevents the DPPH action. The ZnO NPs' antioxidant effectiveness is further revealed by the IC_{50} values, which are shown in (Table 7). With an IC_{50} of 42.21 $\mu\text{g/mL}$, the positive control, conventional ascorbic acid (vitamin C), demonstrated 97.80% inhibition. The substantial antioxidant activity of the produced ZnO NPs was highlighted by their remarkable 67.02 ± 0.00 suppression of the DPPH radicals at highest concentration. Additionally, the ZnO NPs demonstrated strong DPPH radical scavenging; as concentrations were increased from 100 to 500 $\mu\text{g/mL}$, the scavenging percentages increased from 39 to 67%. According to (Khan *et al.*, 2018), un-doped greenly generate ZnO nanoparticles have an IC_{50} value of 51.25 $\mu\text{g/mL}$ and DPPH scavenging action of 60.91% (Al-Rajhi *et al.*, 2022) which is less activity than current study.

Based on the results, ZnO NPs has ability to scavenge DPPH free radicals were significantly enhanced in a dose-dependent manner. This suggests that the ZnO NPs produced using the leaf extract of *Mangifera indica* had a significant level of antioxidant activity. The antioxidant efficacy of ZnO NPs was nonetheless remarkable despite being marginally less than the ascorbic acid reference; this was probably due to the bioactive components that were functionalized on their surface. Due to the absorption and neutralization of free radicals, the quenching of singlet and triplet oxygen and flavonoids capped on the surface of nanoparticles, which have increased antioxidant activity of nanoparticles, the phytochemical constituent of polyphenols exhibits significant antioxidant activity (Vindhya *et al.*, 2023). For Ag-ZnO NCs, the %RSA value ($81.75 \pm 0.00 \mu\text{g/mL}$) and IC_{50} value ($46.50 \mu\text{g/mL}$) were measured at a concentration of 500 $\mu\text{g/mL}$. Therefore, compared to un-doped ZnO nanoparticles, it is clear that doped ZnO nanocomposites containing Ag produced using *Mangifera indica* exhibit enhanced DPPH free radical scavenging ability. The antioxidant activity of the nanocomposite was superior to that of the nanoparticles at all used concentrations of the Ag-ZnO NCs, and a maximum radical scavenging activity (roughly 81%) was noted at the maximum concentration. The results of all concentrations showed that the antioxidant of the nanoparticles and nanocomposite increased with the increased concentration in a dose-dependent manner (Table 7 and 8).

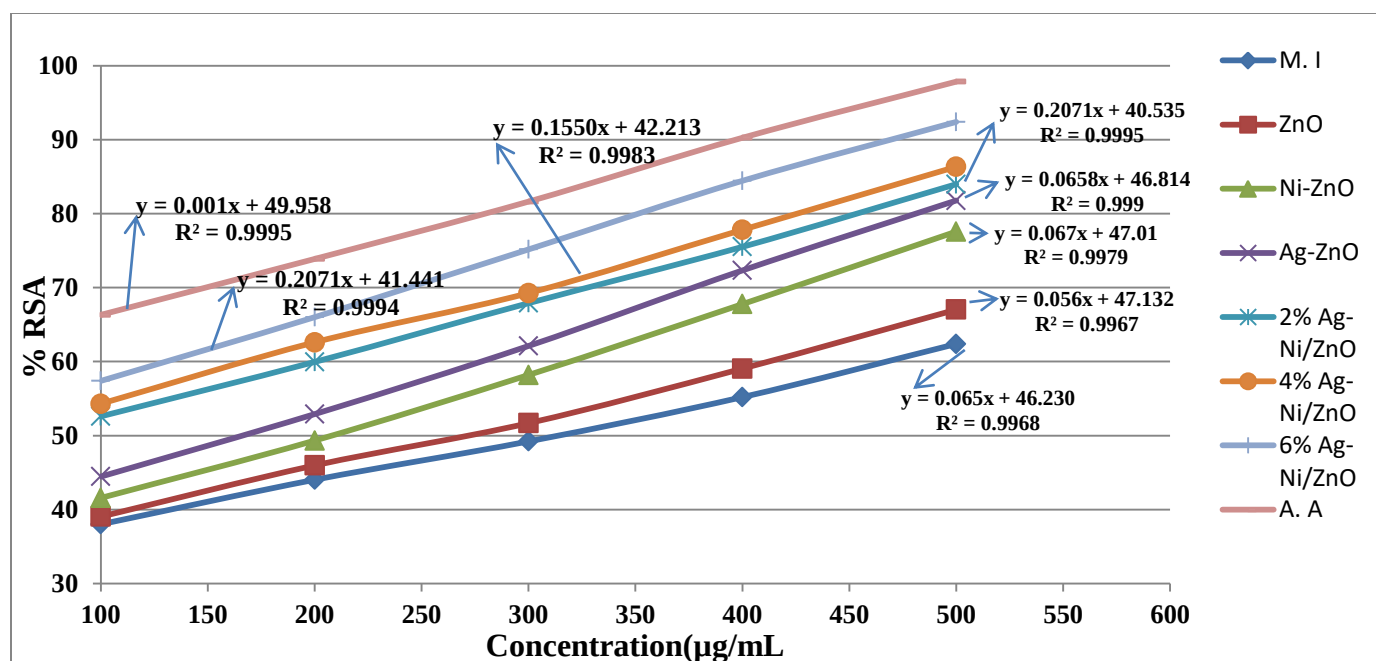


Figure 14. DPPH radical scavenging activity curve for *Mangifera indica* extract, ZnO NPs, Ni-ZnO NCs, Ag-ZnO NCs, Ag-Ni/ZnO NCs (2%, 4%, and 6%), and ascorbic acid.

This discovery indicates that the produced bimetallic nanoparticles may be the fundamental cause of the DPPH free radicals' neutralization by providing electrons. It has previously been reported that adding Ag to the ZnO lattice can increase antioxidant activity. The synergistic effect eliminates free radicals, and the Ag-ZnO NCs made from the leaf extract have functional groups that can improve their antioxidant qualities (Shreema *et al.*, 2021).

The %RSA value for Ni-ZnO NCs was 77.57 ± 0.00 µg/mL, and the IC_{50} value (48.30 µg/mL) was observed at a concentration of 500 µg/mL. Because of Ag-ZnO NCs is stronger synergistic effects and better electrical characteristics than Ni-ZnO NCs, it exhibit higher DPPH free radical scavenging activity than ZnO nanoparticles which agree with (Suresh *et al.*, 2024). The RSA and IC_{50} values for the 2%, 4%, and 6% Ag-Ni/ZnO NCs are $83.98 \pm 0.00\%$, $86.34 \pm 0.05\%$, and $92.36 \pm 0.00\%$, respectively, and their corresponding IC_{50} values are 45.71, 43.62, and 41.30 (Table 7 and 8). The DPPH radicals were shown to be inhibited by the produced nanocomposites by 92.31%, and the highest antioxidant activity at maximum concentration was 41.31 IC_{50} values.

According to the findings, all forms of Ag-Ni/ZnO NCs exhibited increased radical scavenging activity (RSA) as the concentration of nanoparticles increased which is consistent with (Yadesa *et al.*, 2024). Because of the synergistic effects, altered electronic properties, improved stability and dispersion, and enhanced ROS production, the antioxidant activity of Ag-Ni/ZnO NCs (2%, 4%, and 6%) is significantly higher than that of pure ZnO NPs, and the IC_{50} value of 6% Ag-Ni/ZnO NCs was smaller than that of ascorbic acid which is related result as the reported literature (Meshkatsadat *et al.*, 2023). Furthermore, these bioactive chemicals with NCs increase the total antioxidant effectiveness of nanocomposites (Zaater *et al.*, 2024). The straight line indicates that the % scavenging activity rises with increasing concentrations of both the synthesized nanocomposite and the control; however, the nanocomposite has a higher percentage scavenging activity than the nanoparticle. *Mangifera indica* leaves are a source of flavonoids, saponins, and phenolic chemicals, according to a phytochemical investigation. Metal ions in the particles and the chemicals in the leaf extract may have contributed to increase antioxidant activity of biosynthesized NPs and NCs (Khan *et al.*, 2023). The inclusion of Ni^{2+} , Ag^+ , and Zn^{2+} ions in nanoparticles and nanocomposites may be the cause of the increased DPPH free radical scavenging activity.

Table 9. Comparing the effectiveness of the current study with some reported antioxidant activity of green synthesized NPs and NCs.

Sample	Plant extract used	Method used	% RSA	IC ₅₀ (µg/mL)	Refrences
M.I extract Leaf	-	DPPH	Less activity than ZnO NPs	-	Rajeshkumar <i>et al.</i> ,2018
Allium sativum Leaf	-	DPPH	60	-	Benshiga <i>et al.</i> ,2024
ZnO	<i>Mangifera Indica</i> Leaf	DPPH	86	—	Rajeshkumar <i>et al.</i> ,2018
ZnO	<i>Monoon longifolium</i> leave extract	DPPH	78	52	Bekele <i>et al.</i> ,2024
ZnO	<i>Punica granatum L.</i>	DPPH	67	240	Meshkatsadat <i>et al.</i> ,2023
ZnO	<i>Annona Muricata</i> leaf	DPPH	—	25.18	Vindhya <i>et al.</i> ,2023
ZnO	<i>Allium sativum</i> leaf	DPPH	60	—	Benshiga <i>et al.</i> ,2024
ZnO	<i>Ephedra alata</i> leaf	DPPH	70.51	—	Zaater <i>et al.</i> ,2024
ZnO	<i>espresso spent coffee grounds</i>	DPPH	78.07	0.95	Ostovar <i>et al.</i> ,2023
ZnO	<i>Abutilon indicum</i> , <i>Clerodendrum infortunatum</i> , <i>Clerodendrum inerme</i>	DPPH	—	51±0.21	Khan <i>et al.</i> ,2018
SnO ₂	<i>Cleistanthus Collinus</i> leaves	DPPH	59.64	—	Gebreslassie <i>et al.</i> ,2021
Ag-ZnO	<i>Trigonella foenum-graecum</i> leaf	DPPH	—	20.25	Noohpisheh <i>et al.</i> ,2020
Ag-ZnO	<i>Capparis zeylanica</i> leaf extract	DPPH	71.40	340	Suresh <i>et al.</i> ,2024
Ag-ZnO	<i>Morinda citrifolia</i> and leaf	DPPH	83	—	Shreema <i>et al.</i> ,2021
Co-ZnO	<i>Annona Muricata</i> leaf	DPPH	—	15.66	Vindhya <i>et al.</i> ,2023
Cu-NiO	<i>Phytolaccadodecandra L'Herit</i> Leaf	H ₂ O ₂	—	35.29	Firisa <i>et al.</i> ,2022
CuO-GO-Ag	<i>Fagonia arabica</i> leaves	DPPH	59.57	0.35	Shahid <i>et al.</i> , 2022
ZnO	<i>M.indica</i> leaf	DPPH	67.02	51.25	Current study
Ag-ZnO	<i>M.indica</i> leaf	DPPH	81.75	46.50	Current study
Ni-ZnO	<i>M.indica</i> leaf	DPPH	77.57	48.31	Current study
Ag-Ni/ZnO	<i>M.indica</i> leaf	DPPH	92.36	41.30	Current study
M.I extract	-	DPPH	62.38	57.9	Current study

4.6. Antibacterial Activity of nanoparticles and nanocomposites using *Mangifera indica* leaf extract.

Because of their rich phytochemical makeup, plant leaf extracts have antibacterial qualities. For example, leaf extract from *Mangifera indica* inhibits *S. aureus* by 12.31 ± 0.60 mm and *E. coli* by 11.32 ± 0.41 mm. Alkaloids, tannins, flavonoids, and phenols are the compounds that give *Mangifera indica* leaves their antibacterial properties (Maharaj *et al.*, 2022). According to (Zaater *et al.*, 2024), *E. alata* leaf extract has antimicrobial properties. Leaf extract from the *H. abyssinica* plant inhibits *S. aureus* and *E. coli* by 10 ± 0.01 mm and 9.10 ± 0.31 mm, respectively (Zewudie *et al.*, 2023), whereas leaf extract from the *L. cornuta* plant inhibits *S. aureus* and *E. coli* by 7.72 ± 0.50 mm and 7.32 ± 0.51 mm, respectively (Makauki *et al.*, 2023) are some reported sources which assist this result.

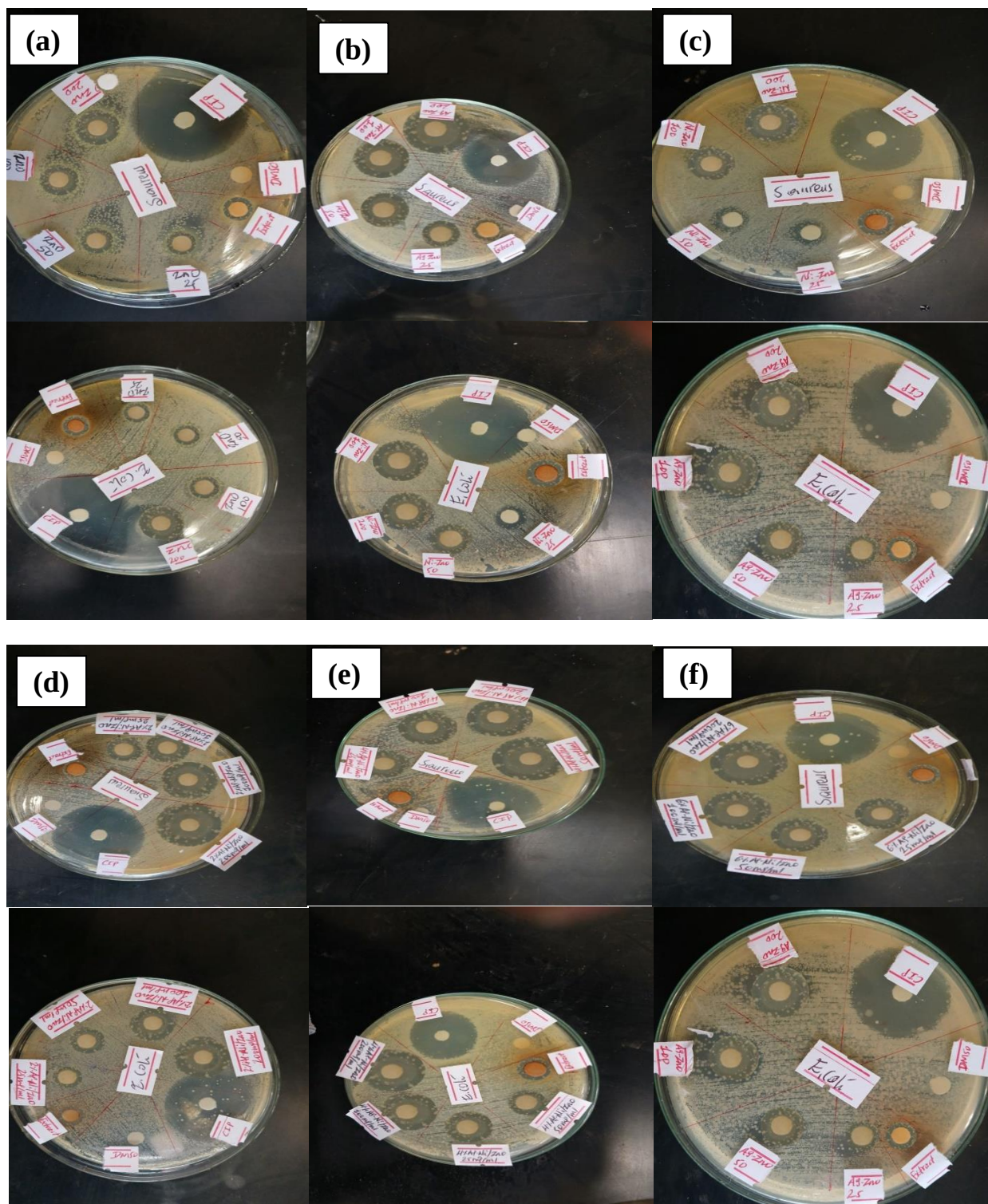
ZnO NPs demonstrated strong antibacterial action against two bacterial strains using this technique (Table 10 and Figure 15). It was shown that a higher ZnO NP concentration gradually inhibits microbial growth. *E. coli* and *S. aureus* showed inhibitory zone diameters of 15.60 ± 0.61 and 15.12 ± 0.43 mm, respectively, at a higher concentration of ZnO NPs (200 mg/mL). For *S. aureus* and *E. coli*, the inhibition zones of ciprofloxacin were 28.30 ± 0.31 mm and 28.31 ± 0.42 mm, respectively. According to the results, ZnO NPs were found to be more efficient against Gram-negative bacteria than Gram-positive ones which agree with (Ihsan *et al.*, 2023). This result was in close agreement with ZnO NPs with ZOI of 15.00 mm and 14.67 mm for *E. coli* and *S. aureus* respectively (Ihsan *et al.*, 2023). Other reported literature support this result as ZnO nanoparticles against *B. subtilis* was mild and exhibited a zone of inhibition (ZOI) of 14.33mm and against *E. coli*, the antibacterial activity of ZnO NPs (ZOI = 15.13 mm) (Kumar *et al.*, 2019) and against *S. aureus* and *E. coli* exhibited a zone of inhibition (ZOI) of 10.51 mm and 12.00 mm respectively (Rashid *et al.*, 2024). (Makauki *et al.*, 2023) reported that ZnO NPs had a ZOI of 11.01 ± 0.80 mm and 7.70 ± 0.90 mm for *E. coli* and *S. aureus*, respectively. ZnO NPs showed more antibacterial activity against *S. aureus* than against *E. coli*, and the antibacterial activities were concentration-dependent which was in close agreement with (Bekele *et al.*, 2024). This resulted from the different composition of the bacteria's cell walls. Gram-negative bacteria are more resistant to ZnO NPs than Gram-positive bacteria because they have lipopolysaccharide molecules on their outer membrane layer, which illustrates the responsiveness of gram-positive and gram-negative bacteria.

Table 10. Zone of inhibition of synthesized ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, and Ag-Ni/ZnO NCs (2%, 4%, and 6%) using *Mangifera indica* leaf extract against *S. aureus* and *E. coli*.

S/N	Sample	Dose(mg/mL)	Zone of Inhibition(mm)	
			<i>S.aureus</i>	<i>E.coli</i>
1	ZnO	200	15.6±0.61	15.1±0.42
		100	10.3±0.73	13.3±0.91
		50	9.7±0.40	11.6±0.44
		25	9.9±0.53	10.2±0.35
2	Ag-ZnO	200	21.0±0.83	18.0±0.82
		100	17.0±0.80	16.6±0.43
		50	14.0±0.84	13.6±0.94
		25	11.3±0.62	10.3±0.45
3	Ni-ZnO	200	16.0±0.83	16.3±0.41
		100	14.0±0.81	15.0±0.80
		50	10.0±0.80	12.0±0.70
		25	10.3±0.70	8.6±0.93
4	2%Ag-Ni/ZnO	200	22.1±0.43	19.6±0.31
		100	15.6±0.41	19.0±0.84
		50	12.3±0.54	15.0±0.85
		25	10.0±0.43	13.6±0.40
5	4%Ag-Ni/ZnO	200	22.6±0.83	19.8±0.42
		100	19.3±0.91	17.5±0.83
		50	17.3±0.42	14.3±0.42
		25	14.5±0.80	11.0±0.41
6	6%Ag-Ni/ZnO	200	23.4±0.4	20.0±0.64
		100	20.3±0.74	17.7±0.93
		50	17.7±0.55	15.6±0.42
		25	16.4±0.63	12.7±0.31
7		M.I extract	10.2±0.40	10.1±0.42
8		Ciprofloxacin	30.0±0.32	31.7±0.40
9		DMSO	0	0

The generation of ROS, the generation of peroxide anions and superoxide radicals, and the internalization of nanoparticles into bacterial cell walls are other potential sources of cell injury. The disk technique results for Ag-ZnO NCs showed an inhibition zone diameter of 18.00±0.81 mm for *Escherichia coli* and 21.01±0.80 mm for *Staphylococcus aureus*. According to the findings, *E. coli* was less affected by Ag-ZnO nanocomposites than *S. aureus*.

For *S. aureus* and *E. coli*, the inhibition zones for ciprofloxacin were 31.6 ± 0.4 mm and 32.31 ± 0.42 mm, respectively. Gram-negative bacteria are therefore less vulnerable to antibiotic substances which are supported by (Noohpisheh *et al.*, 2020). Other reported sources of Ag-ZnO NCs with ZOI values of 20.33 mm against *E. coli* and 24.27 mm against *S. aureus* (Kumar *et al.*, 2019) and 29.00 mm and 22.01 mm, respectively, against *E. coli* and *S. aureus* (Pandiyan *et al.*, 2019) are comparable to the current study. The ciprofloxacin inhibition zones for *S. aureus* and *E. coli* were 31.61 ± 0.40 mm and 32.32 ± 0.41 mm, respectively. By binding to the thiol groups of proteins and enzymes, Ag^+ prevents DNA replication and enzyme function. Moreover, Ag^+ can be absorbed and generate reactive oxygen species (ROS), causing oxidative stress that alters the cell cycle and encourages cell death by apoptosis or autophagy. Furthermore, it has been demonstrated that Ag^+ binds to the sulfhydryl groups of the metabolic enzymes of the bacterial electron transport chain, rendering them inactive. Several studies indicate that Ag-ZnO nanocomposites exhibit more potent antibacterial properties than ZnO nanoparticles (Makauki *et al.*, 2023).



They were 16.30 ± 0.42 mm for Ni-ZnO NCs and 16.00 ± 0.81 mm for *S. aureus* and *E. coli*, respectively. For *S. aureus* and *E. coli*, the inhibition zones for ciprofloxacin were 31.00 ± 0.72 mm and 34.01 ± 0.80 mm, respectively. The reported Ni-ZnO NCs against *S. aureus* and *E. coli* were 25.01 mm and 27.00 mm, respectively (Jebessa *et al.*, 2017) and the ZOI of Ni-ZnO NCs against *S. aureus* and *E. coli* were 13.00 mm and 12.00 mm, respectively (Faye *et al.*, 2022) are other related reported literature to current result. According to (Table 10), the Ni-ZnO in this investigation exhibited the highest growth inhibitory efficacy against *E. coli* which is in close agreement with (Naseer *et al.*, 2022). Larger radius ionic metal doping has the potential to produce a number of electronic defects in the ZnO band gap, which are essential to the antibacterial action. This could imply that the antibacterial activity of ZnO is significantly influenced by the electrical flaws in its band gap. According to (Jebessa *et al.*, 2017), Ni-ZnO NCs showed a greater inhibitory zone against gram negative bacteria than gram positive bacteria which is similar to current study. Ni-ZnO shown the highest growth inhibitory efficacy against *E. coli* (Naseer *et al.*, 2022), while (Naskar *et al.*, 2020) noted a similar pattern.

For 2% Ag-Ni/ZnO NCs, against *E. coli* and *S. aureus*, they were 22.10 ± 0.40 mm and 19.61 ± 0.31 mm, respectively; for 4% Ag-Ni/ZnO NCs, against *E. coli* and *S. aureus*, they were 19.81 ± 0.42 mm and 22.60 ± 0.81 mm, respectively; for 6% Ag-Ni/ZnO NCs, against *E. coli* and *S. aureus*, they were 20.01 ± 0.60 mm and 23.40 ± 0.43 mm, respectively (Table 10).

All monodoped NCs and codoped NCs have better antibacterial activities than pure ZnO NPs because of increased ROS and the number of ions added as a dopant to the host metal oxide (ZnO), which helps to inhibit bacteria. The greatest concentration of 6% Ag-Ni/ZnO NCs produced the largest inhibition zone (23.42 ± 0.41 mm) *S. aureus*. According to the results, ZnO NPs, Ag-ZnO NCs, and Ag-Ni/ZnO NCs were found to be more efficient against Gram-positive bacteria than Gram-negative bacteria. This resulted from the bacteria's differing cell wall compositions; compared to Gram-positive bacteria, Gram-negative bacteria are more resistant to NPs and NCs because they have lipopolysaccharide molecules on their outer membrane layer. (Ostovar *et al.*, 2023) and (Thakur *et al.*, 2022) noted similar patterns. The adsorption of the phytochemical component of *Mangifera indica* leaf extract on NCs, as well as enhanced stability as a result of doping, which makes it easier for nanomaterials to pass through bacterial cell walls, is responsible for the increase in antimicrobial activity. This alters the cytoplasmic membrane's structure, causing organelles to burst and possibly even cell lysis (Yadesa *et al.*, 2024).

Ag-Ni/ZnO NCs developed more oxygen vacancies, which increased the amount of ROS and the synergistic effect of codoped NCs over metal oxide NPs. Monodoped NCs also increased the antibacterial activity of the Ag-Ni/ZnO NCs. The negatively charged microbial cell membranes come into touch with $\text{Ag}^+/\text{Ni}^{2+}/\text{Zn}^{2+}$ ions that are released by Ag-Ni/ZnO NCs which boost antibacterial activity (Thakur *et al.*, 2022). Similarly, (Pandiyar *et al.*, 2019) found that the positive charge of Ag^+ , Au^+ , and Zn^{2+} NCs interacts electrostatically with the negative charge of the bacterial cell membrane to produce reactive oxygen species (ROS), which primarily kills the bacteria.

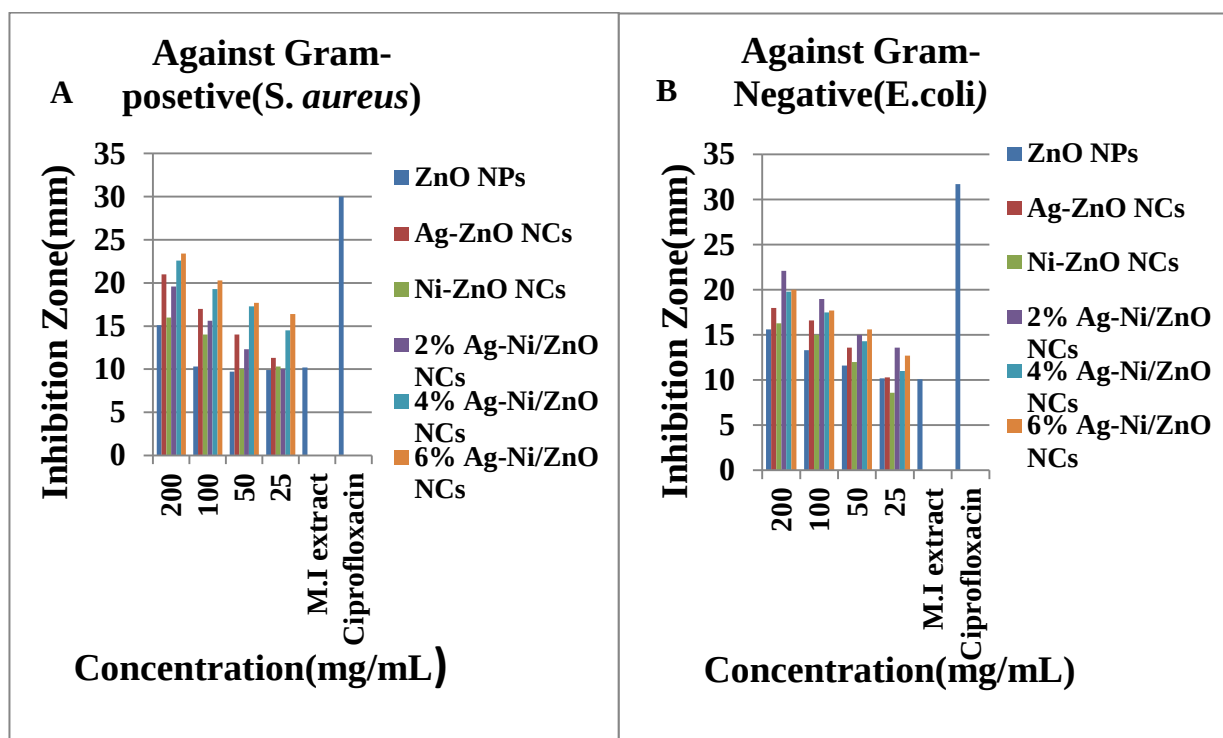


Figure 16. Bar graph representation of antibacterial activity for ZnO NPs, Ni-ZnO NCs, Ag-ZnO NCs, and Ag-Ni/ZnO NCs (2%, 4%, and 6%) of A) *S. aureus* and B) *E. coli*.

Furthermore, Ag^+ and Ni^{2+} ions in the ZnO lattice increase the density of oxygen vacancies and defects, which enhances the antibacterial activity of ZnO NPs doped with silver and nickel. Therefore, in order to provide superior antibacterial activity over pure ZnO NPs, the release of metal ions and the production of ROS may break down the bacterial cell wall. According to (Pandiyar *et al.*, 2019), ZnO Nps codoped with silver and nickel show superior antibacterial activity and as concentration grew; the antibacterial activity of all samples is improved even more which is similar pattern with current study.

Table 11. Comparing the current study with some reported antibacterial activity of green synthesized NPs and NCs.

Sample	Plant extract used	Types of Bacteria used	Inhibition zone(mm)	References
ZnO	<i>Monoon longifolium</i> leave	<i>S aureus</i>	22 ± 0.57	Bekele <i>et al.</i> ,2024
ZnO	<i>espresso spent coffee grounds</i>	<i>E. coli</i> and <i>S aureus</i>	22±0.25, 28±0.38	Ostovar <i>et al.</i> ,2023
Ag-ZnO	<i>Colocasia esculenta</i> leaf	<i>B.Subtilis</i> and <i>E. coli</i>	14.33, 15.13	Kumar <i>et al.</i> ,2019
Co-ZnO	<i>Annona Muricata</i> leaf	<i>s.aureus</i> , <i>p.aeruginosa</i> , <i>b .subtilis</i>	13, 13, 14	Vindhya <i>et al.</i> ,2023
Ni-ZnO	<i>Aloe vera</i> leaf	<i>E.Coli</i> , <i>Klbsiella Pneumoniae</i> , <i>S. aureus</i> and <i>Streptococcus pyogenes</i> .	<i>E.Coli</i> > <i>K. Pneumoniae</i> > <i>S. aureus</i> > <i>S. pyogenes</i> .	Mary <i>et al.</i> ,2022
Ag-ZnO	<i>Trigonella foenum-graecum</i> leaf	<i>E. coli</i> and <i>S aureus</i>	12.5±0.707, 13.5±0.707	Noohpisheh <i>et al.</i> ,2020
Ni-ZnO	<i>UsingEuphorbia abyssinica</i> bark	<i>s.aureus</i> , <i>E.coli</i> , <i>B.subtitles</i> and <i>aspergillum</i>	13,12,13,13	Faye <i>et al.</i> ,2022
Ag-ZnO	<i>Macrotyloma Uniflorum</i> Leaf	<i>B.subtilis</i> , <i>Streptococci</i> and <i>E.coli</i>	12±1.5, 11±0.6, 8±0.3	Sali <i>et al.</i> ,2021
Ag-ZnO	<i>Launaea cornuta</i> Leaf	<i>E. coli</i> and <i>S aureus</i>	21 ± 1.1, 19.7 ± 0.5	Makauki <i>et al.</i> ,2023
Se-ZnO	<i>Mangifera indica</i> Leaf	<i>E. coli</i> and <i>S aureus</i>	18,13	Dhivya <i>et al.</i> ,2019
Ag-Au/ZnO	<i>J.adhatoda</i> leaves	<i>E. coli</i> and <i>S aureus</i>	34,26	Pandiyan <i>et al.</i> ,2019
Ag-Cu/ZnO	<i>Aloe vera gel</i>	<i>P. aeruginosa</i> , <i>B.Subtilis</i> , <i>S aureus</i>	12,9,-	Rashid <i>et al.</i> ,2024
Ag-Mo/CuO	<i>Azadirachta indica</i> leaf	(<i>S.aureus</i> , <i>Serratia marcescens</i> , <i>Candida albicans</i> , <i>P.aeruginosa</i>)	17,15,17,16,	Rajendaran <i>et al.</i> ,2019
ZnO	<i>M.indica</i> Leaf	<i>S aureus</i> , <i>E. coli</i>	15.61±0.60, 15.10±0.41	Current study
Ag-ZnO	<i>M.indica</i> Leaf	<i>S aureus</i> , <i>E. coli</i>	21.00±0.81, 18.01±0.82	Current study
Ni-ZnO	<i>M.indica</i> Leaf	<i>S aureus</i> , <i>E. coli</i>	16.02±0.81, 16.30±0.43	Current study
Ag-Ni/ZnO	<i>M.indica</i> Leaf	<i>S aureus</i> , <i>E. coli</i>	23.40±0.43, 20.00±0.62	Current study

4.7. Possible antibacterial mechanisms of nanoparticles and nanocomposites.

Antibacterial mechanisms of NPs and NCs are explained differently by different authors. Therefore, three mechanisms account for ZnO antibacterial activity NPs: the generation of reactive oxygen species (ROS) through electrostatic interaction with cell membranes, which can react with DNA, membranes, proteins, and lipids to cause cell death; the loss of cell membrane due to the release of zinc ions and their accumulation in the cell; and the direct contact and penetrability of ZnO NPs to the bacterial cell wall, which kills the bacterial cell (Ostovar *et al.*, 2023). The antibacterial mechanism of Ag-ZnO NCs entails direct interaction with the bacterial membrane via either i) complicated porosity, ii) electrostatic interaction between the released ions and the negatively charged cell wall, or iii) silver and zinc ions released following surface oxidation. Silver (Ag) and zinc oxide (ZnO) nanoparticles work in concert in Ag-ZnO. Furthermore, Ag^+ has the ability to be internalized and produce reactive oxygen species (ROS), which results in oxidative stress that modifies the cell cycle and promotes cell death via autophagy or apoptosis. Ag possesses potent antimicrobial properties due to its ability to release silver ions (Ag^+) that can disrupt bacterial cell membranes and inhibit essential cellular functions, resulting in the cellular death of affected bacteria (Makauki *et al.*, 2023).

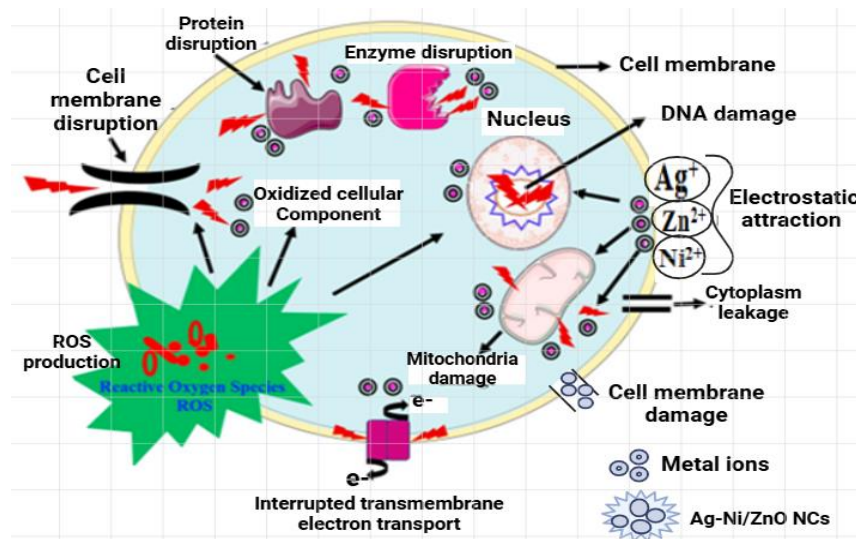


Figure 17. Proposed Antibacterial inhibition mechanism of Ag-Ni/ZnO NCs.

Doping metal with larger radius ionic metals like Ni-ZnO NCs may introduce various electronic defects in the ZnO band gap that play a crucial role in the antibacterial mechanism, and this may suggest that the electronic defects in the ZnO band gap play a crucial role in its antibacterial activity (Jebessa *et al.*, 2017). More oxygen vacancies were observed in the Ag-Ni/ZnO NCs, resulting in a higher amount of ROS and a greater synergetic effect of codoped NCs than metal oxide NPs and monodoped NCs, which boosts antibacterial activity (Thakur *et al.*, 2022). On the other hand, Ag^+ / Ni^{2+} / Zn^{2+} metal ions released by Ag-Ni/ZnO NCs NCs come in contact with negative charge of microbial cell membranes which penetrate and hook onto the cell membrane and react with sulfhydryl groups within the cell membrane, which results in the cell death of the bacteria (Elderderly *et al.*, 2023).

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The research aims to establish *Mangifera indica* leaf as a selected in environmentally friendly synthesis of Ag-Ni/ZnO NCs and investigate their antioxidant and antibacterial performance. The effects of dopant concentration, volume, pH, and temperature were optimized before synthesis. The physical examination of *mangifera indica* leaf extract revealed the presence of phenolic compounds, saponins, alkaloid, tannin, and flavonoid. The band gap energy (E_g) was computed from the Tauc plot using the Tauc/DavidMatt model after the electronic spectra of the ZnO NPs, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO NCs were recorded in the 200–800 nm range using a UV-Vis spectrophotometer. UV-Vis spectra of *Mangifera indica* leaf extract revealed a peak at 260 and 278 nm, which was caused by the conjugated C=C bond $\pi-\pi^*$ transition in aromatic rings. The presence of ZnO nanoparticles is indicated by an absorption peak at 354 nm in the UV-Vis spectrophotometric data. With band gap energy of 2.83 eV, the absorption peak of Ag-ZnO NCs was detected at 363 nm. The band structure of ZnO nanoparticles has changed, causing a shift towards higher wavelengths and a decrease in the band gap. An absorption band around 358 nm corresponds to Ni-ZnO NCs is due to free excitons. The UV-Vis spectroscopic data for Ag-Ni/ZnO NCs showed absorption wavelengths of 360, 362, and 366 nm, respectively. The band gap of Ag-Ni/ZnO decreased to 3.02, 2.92, and 2.72 eV for 2% Ag-Ni/ZnO, 4% Ag-Ni/ZnO, and 6% Ag-Ni/ZnO NCs, respectively. The energy band and absorption edge of co-doped ZnO NCs showed a red shift, indicating visible-light harvesting ability.

The presence of functional groups and the chemical bond between greenly synthesized ZnO nanoparticles, Ag-ZnO, Ni-ZnO, and Ag-Ni/ZnO nanocomposites were determined using Fourier transform infrared (FT-IR) spectra recorded at 4000-400 cm^{-1} . The analysis revealed the presence of metal oxygen bonds (M-O) in the Ag-Ni/ZnO nanocomposites as well as the presence of various phytochemicals in the leaf extract of *Mangifera indica*, which was utilized as a capping and reducing agent. X-ray diffraction (XRD) in the 2θ range of 20 to 80 was used to assess the crystalline phase and crystallinity of the synthesized samples, and the Debye-Scherrer equation was used to establish the average crystallite size. All ZnO NPs, monodoped NPs and codoped NCs show the hexagonal wurtzite crystal structure.

In Ag-ZnO NCs, the three extra diffraction peaks (111), (200), and (220) are associated with the face-centered cubic (fcc) structure of silver. The significant difference in ionic size between Ag^+ and Zn^{2+} ions in the ZnO NPs system is the cause of this. With the exception of a few changes in diffraction angle and intensity, Ni-ZnO NCs exhibit comparable diffraction patterns. The size trend goes as follows: Because the dopant has a wider ionic radius than the host material, ZnO NPs < Ni-ZnO NCs < Ag-ZnO NCs < 2% Ag-Ni/ZnO NCs < 4% Ag-Ni/ZnO NCs < 6% Ag-Ni/ZnO NCs. The study examined a capacity of synthesized nanocomposites to scavenge free radicals at different concentrations. The results showed that *Mangifera indica* leaf extract and ascorbic acid scavenged free radicals found in DPPH due to the presence of a major bioactive compound. The synthesized ZnO nanoparticles demonstrated significant antioxidant activity, with IC_{50} %RSA values of 67.02 ± 0.00 . The %RSA value for Ag-ZnO NCs and Ni-ZnO NCs were $81.75 \pm 0.00 \mu\text{g/mL}$ and $77.57 \pm 0.00 \mu\text{g/mL}$, respectively. The nanocomposites demonstrated 92.31 % inhibition of DPPH radicals, with IC_{50} values of 41.30 for 6% Ag-Ni/ZnO NCs. Compared to pure ZnO NPs, Ag-Ni/ZnO NCs exhibited much higher antioxidant activity. With a maximal ZOI of 23.4 ± 0.4 for Ag-Ni/ZnO NCs and 15.6 ± 0.61 for ZnO NPs, the NCs and NPs start the production of reactive oxygen species (ROS) and increased ions. Because Gram-positive and Gram-negative bacteria have distinct cell wall compositions, ZnO nanoparticles, Ag-ZnO nanocomposites, and Ag-Ni/ZnO nanocomposites were more efficient against Gram-positive bacteria. This study showed that, the use of *Mangifera indica* leaf extract for green syntheses of nanomaterials have an advance for biomedical applications as a eco-friendly and cost-effective techniques.

5.2. Recommendation

The study should take into account the following recommendations in light of current findings.

- ✓ In order to examine size, morphology, elemental distribution and chemical composition of nanocomposite sample should be characterized by TEM, SEM, EDX and XPS instrument.
- ✓ Additionally, the application of the material should include the photocatalytic degradation, antifungal, anticancer, antiplasmodial, and anti-inflammatory properties are applications that may be of interest for future research work.
- ✓ In vivo antioxidant and antibacterial analysis should be done using NPs and NCs.
- ✓ It is easy to maintain the appropriate conditions for the synthesis of NPs and NCs from plants at the small-scale laboratory level but large scale production is a challenge so further study should be carried out to solve this problem.
- ✓ Using other *mangifera indica* plant part for the synthesis of nanocomposites should be the main focus of future work.

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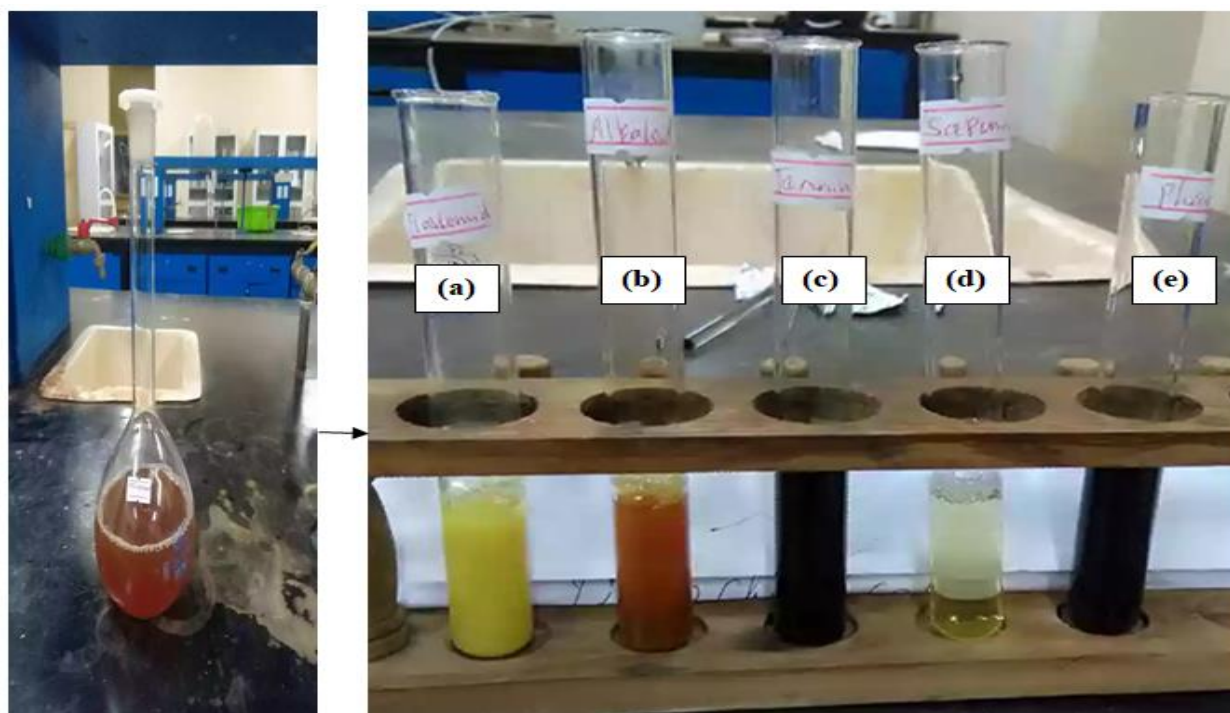
7. APPENDICES

Appendix 1. Percent by mass and molarity of dopant (nickel nitrate hexahydrate and silver nitrate) with zinc sulfate heptahydrates by using calculated volume ratio method.

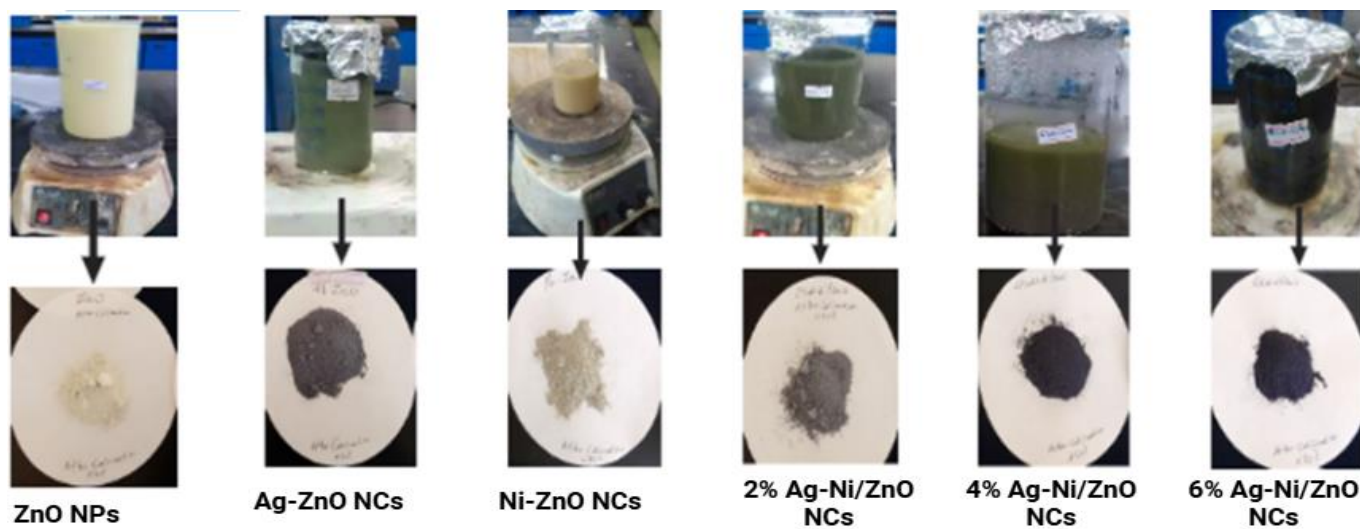
% of Ag and Ni	mass(Ni)	M(Ni(NO ₃) ₂ · 6H ₂ O)	mass(Ni(NO ₃) ₂ · 6H ₂ O)	mass(Ag)	M(AgNO ₃)	mass(AgNO ₃)	M(ZnSO ₄ · 7H ₂ O)
1%	0.0058	0.001	0.02907	0.0107	0.001	0.01699	0.098(2.81g)
2%	0.01174	0.002	0.05814	0.02156	0.002	0.03398	0.096(2.76g)
3%	0.01761	0.003	0.0872	0.03234	0.003	0.05097	0.094(2.70g)
4%	0.02348	0.004	0.01162	0.04312	0.004	0.0679	0.092(2.64g)
5%	0.02935	0.005	0.1453	0.0539	0.005	0.08495	0.090(2.58g)
6%	0.03522	0.006	0.1740	0.6467	0.006	1.019	0.088(2.53g)



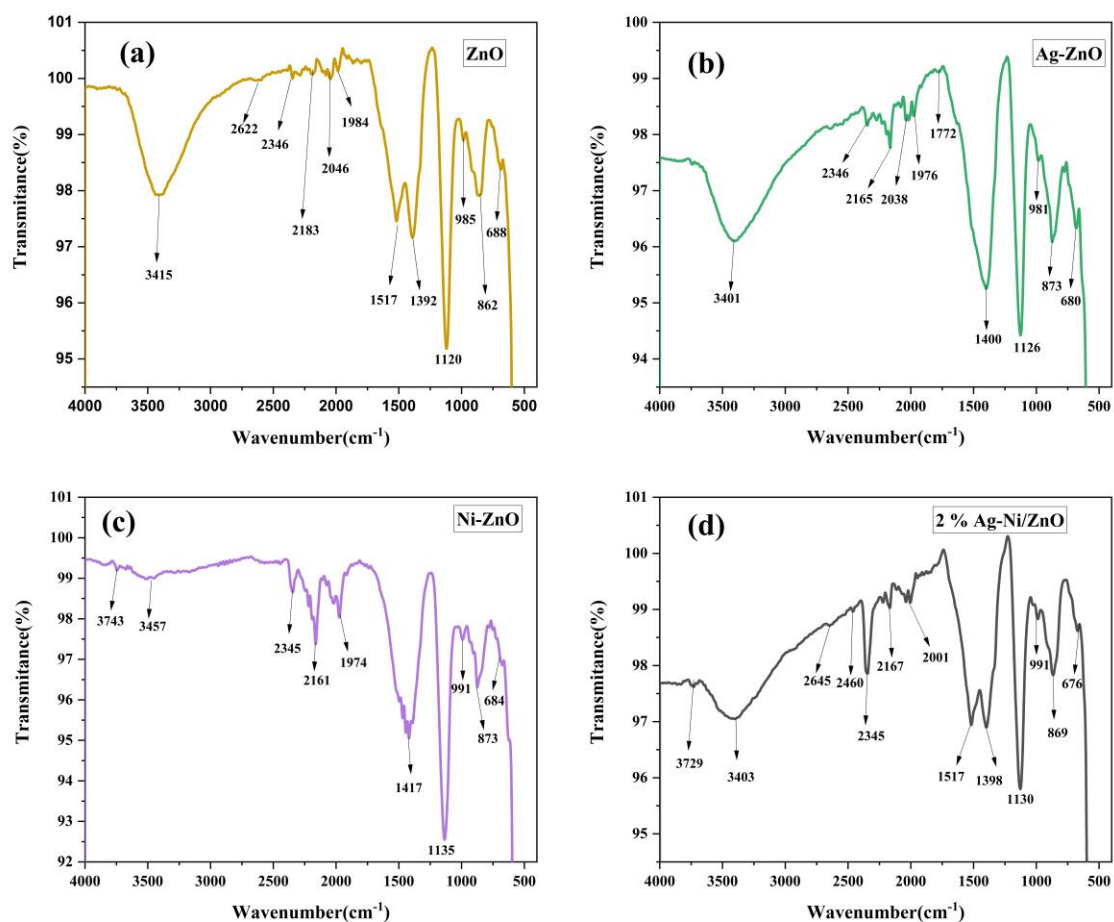
Appendix 2. Preparation process of *Mangifera indica* leaf extract.



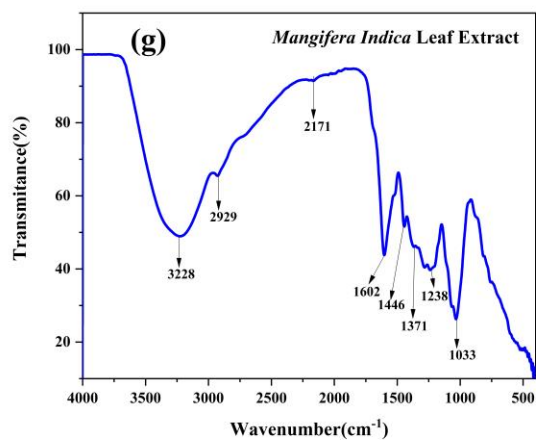
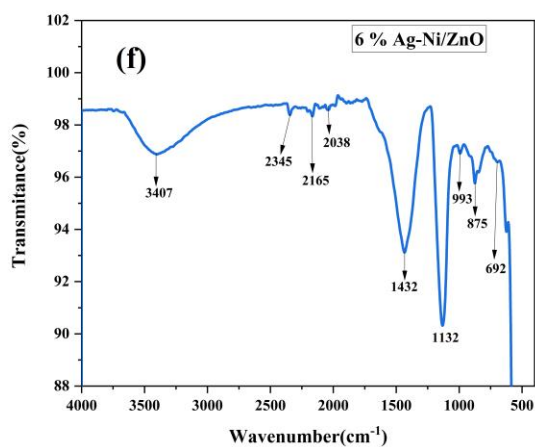
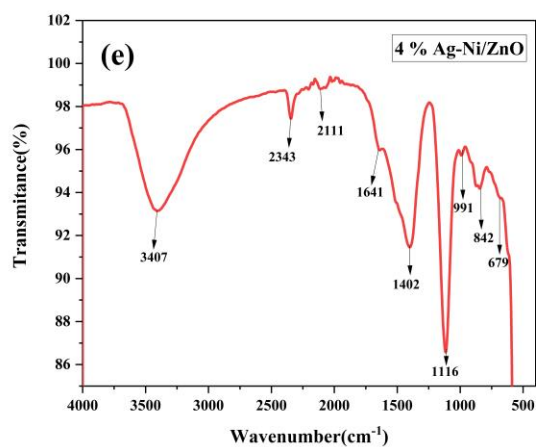
Appendix 3. Phytochemical screening test results for flavonoid (a), alkaloid (b), tannin(c), saponin (d) and phenol (e) of *Mangifera indica* leaf extract.



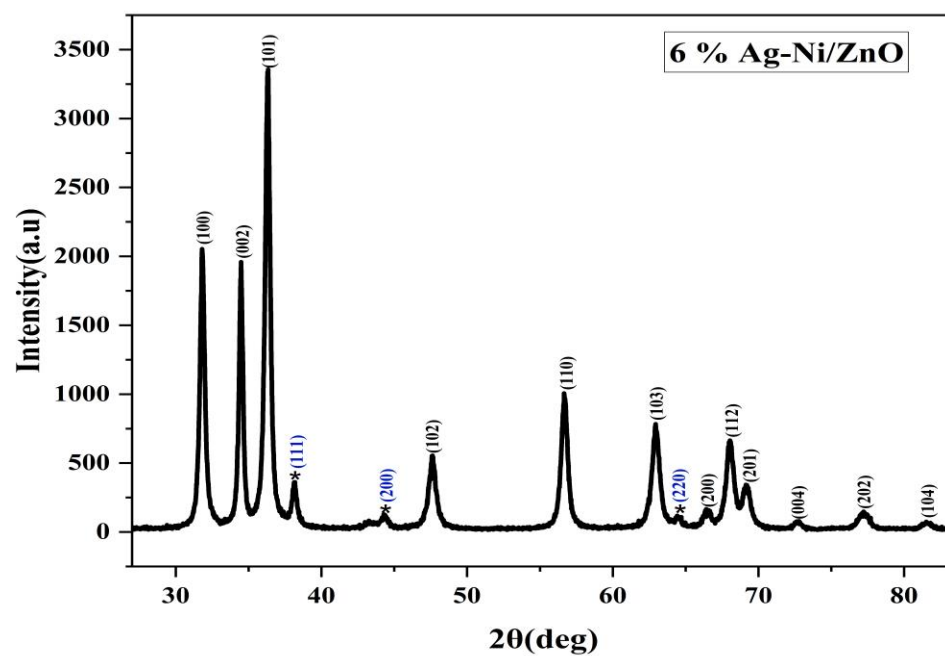
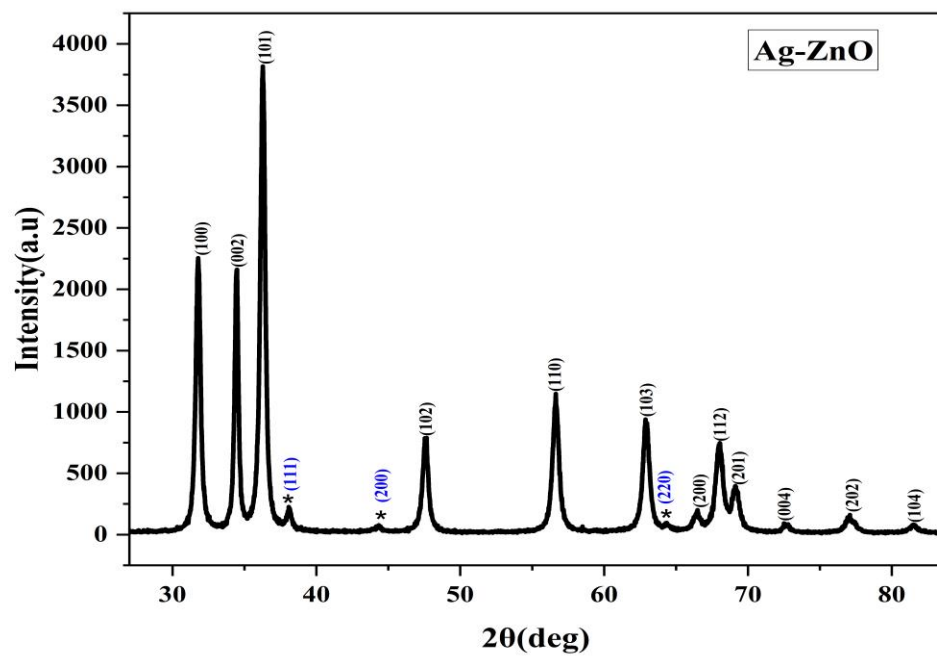
Appendix 4. Figurative description of synthesized ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs and Ag-Ni/ZnO NCs (2%, 4% and 6%).



Appendix 5. FTIR Spectral absorption band of ZnO NPs, Ag-ZnO NCs, Ni-ZnO NCs, 2% Ag-Ni/ZnO NCs and *Mangifera indica* leaf extract.



Appendix 6. FTIR Spectral absorption band of 4% Ag-Ni/ZnO NCs, 6% Ag-Ni/ZnO NCs and *Mangifera indica* leaf extract.



Appendix 7. XRD graphs of synthesized Ag-ZnO NCs and 6% Ag-Ni/ZnO NCs